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35th Edition

CLSI M100™

Performance Standards for Antimicrobial Susceptibility Testing

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A CLSI supplement for global application.

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CLSI M100-Ed35
January 2025
Replaces CLSI M100-Ed34

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Abstract

The data in the tables are valid only if the methodologies in CLSI M02,[1] M07,[2] and M11[3] are followed. These standards contain information about disk diffusion (CLSI M02[1]) and dilution (CLSI M07[2] and CLSI M11[3]) test procedures for aerobic and anaerobic bacteria. Clinicians depend heavily on information from the microbiology laboratory for treating their seriously ill patients. The clinical importance of antimicrobial susceptibility test results demands that these tests be performed under optimal conditions and that laboratories have the capability to provide results for the newest antimicrobial agents. The tables presented in CLSI M100 represent the most current information for drug selection, interpretation, and quality control using the procedures standardized in CLSI M02,[1] M07,[2] and M11.[3] Users should replace previously published tables with these new tables. Changes in the tables since the previous edition appear in boldface type.

Clinical and Laboratory Standards Institute (CLSI). *Performance Standards for Antimicrobial Susceptibility Testing.* 35th ed. CLSI supplement M100 (ISBN 978-1-68440-262-5 [Print]; ISBN 978-1-68440-263-2 [Electronic]). Clinical and Laboratory Standards Institute, USA, 2025.

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Suggested Citation

CLSI. *Performance Standards for Antimicrobial Susceptibility Testing.* 35th ed. CLSI supplement M100. Clinical and Laboratory Standards Institute; 2025.

Previous Editions:

December 1986, December 1987, December 1991, December 1992, December 1994, December 1995, January 1997, January 1998, January 1999, January 2000, January 2001, January 2002, January 2003, January 2004, January 2005, January 2006, January 2007, January 2008, January 2009, January 2010, June 2010, January 2011, January 2012, January 2013, January 2014, January 2015, January 2016, January 2017, January 2018, January 2019, January 2020, March 2021, February 2022, March 2023, February 2024

CLSI M100-Ed35
ISBN 978-1-68440-262-5 (Print)
ISBN 978-1-68440-263-2 (Electronic)
ISSN 1558-6502 (Print)
ISSN 2162-2914 (Electronic)

Volume 45, Number 1

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Acknowledgment

CLSI and the Subcommittee on Antimicrobial Susceptibility Testing gratefully acknowledge the following volunteers for their important contributions to the revision of CLSI M100:

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Overview of Changes

CLSI M100-Ed35 replaces CLSI M100-Ed34, published in 2024. Major additions, reformatting, and/or table relocation changes are summarized below, followed by additional noteworthy changes detailed by section/table. Changes to content since the previous edition appear in boldface type; however, minor editorial or formatting changes are not listed here, nor highlighted in boldface type. To learn more about the organization of CLSI M100-Ed35, check the "Instructions for Use."

CLSI M100 is updated and reviewed annually as new data and new agents become available. Use of outdated documents is strongly discouraged.

Major Additions and/or Revisions
• Throughout: Changed categorization of disk diffusion from a "reference" method to a "standard" method; the disk diffusion method described in CLSI M02[1] is no longer considered a reference method but remains a standard method. • Throughout: Modified QC testing frequency recommendations from "daily or weekly" to "daily or per IQCP." • Tables 1: Removed all footnotes related to testing tetracycline and extrapolating results for doxycycline and/or minocycline (Tables 1A-1, 1A-2, 1B-2, 1B-5, 1C, 1D, 1E, 1G, and 1H-1); these comments are retained in the respective Tables 2 where relevant. • Tables 2: Changed title of "Routine QC Recommendations" box to "QC Recommendations" and removed listings of specific QC strains from the boxes; recommendations for QC strain testing and frequency are now in Appendix I. • Tables 1 and 2: Removed fluoroquinolones from the "Warning" box that lists agents that should not be reported on CSF isolates. • Tables 2: Modified comments related to testing tetracycline and extrapolating results for doxycycline and/or minocycline, as appropriate for organisms or organism groups where tetracycline, doxycycline, and/or minocycline breakpoints are listed. • Table 2A-1, Table 3B, and Table 3C: Enhanced recommendations for the performance of carbapenemase testing, including the identification of the carbapenemase type, for carbapenem-resistant Enterobacterales to support treatment decisions and infection control practices. • Table 2B-3 and Appendix F: Removed MIC breakpoints which are no longer considered reliable for *Burkholderia cepacia* complex. Added instructions for handling *B. cepacia* complex should AST be requested. Developed ECVs for *B. cepacia* complex and added these to Appendix F. • Appendix H: Expanded to include testing instructions when an MIC method for any agent is modified beyond the standard CLSI MIC reference method. Added method for testing exebacase (Appendix H2) that includes the instructions for testing exebacase previously located in Tables 5A-1 and 6A. • Appendix I: Added new appendix with suggestions for development of a QC plan that includes selection of QC strains and QC testing frequency.

Overview of Changes (Continued)

Section/Table	Changes
General	
CLSI Breakpoint Revisions Since 2010	**Revised:** • Ampicillin-sulbactam disk diffusion breakpoints for *Acinetobacter* spp. • Minocycline disk diffusion and MIC breakpoints for *Acinetobacter* spp. ***Deleted:*** • Doxycycline disk diffusion and MIC breakpoints for *Acinetobacter* spp. • Tetracycline disk diffusion and MIC breakpoints for *Acinetobacter* spp. • Ceftazidime MIC breakpoints for *B. cepacia* complex • Chloramphenicol MIC breakpoints for *B. cepacia* complex • Levofloxacin MIC breakpoints for *B. cepacia* complex • Meropenem MIC breakpoints for *B. cepacia* complex • Minocycline MIC breakpoints for *B. cepacia* complex • Ticarcillin-clavulanate MIC breakpoints for *B. cepacia* complex • Trimethoprim-sulfamethoxazole MIC breakpoints for *B. cepacia* complex
CLSI Archived Resources	***Deleted:*** • Table with links to archived resources (the archived resources remain on the CLSI website)
Instructions for Use of Tables	***Deleted:*** • Fluoroquinolones from the CSF warning box
Tables 1. Antimicrobial Agents That Should Be Considered for Testing and Reporting by Microbiology Laboratories	
Table 1A-1. Enterobacterales (excluding *Salmonella* and *Shigella* spp.)	**Added:** • Footnote d regarding cascade reporting rules for aztreonam
Table 1B-3. *Burkholderia cepacia* Complex	**Added:** • Comment regarding location of information for testing *B. cepacia* complex ***Deleted***: • All antimicrobial agents for testing and reporting: – Ceftazidime – Levofloxacin – Meropenem – Minocycline – Trimethoprim-sulfamethoxazole

Overview of Changes (Continued)

Section/Table	Changes
Tables 1. (Continued)	
Table 1J. Anaerobes	**Revised:** • Footnote c regarding penicillin testing and the presence of β-lactamases
Tables 2. Zone Diameter and/or MIC Breakpoints	
Table 2A-1. Zone Diameter and MIC Breakpoints for Enterobacterales (excluding *Salmonella* and *Shigella* spp.)	**Revised:** • Comment regarding carbapenem testing for Enterobacterales • Comment regarding tetracycline susceptibility prediction for doxycycline and minocycline susceptibility ***Deleted:*** • Comment regarding sulfisoxazole to represent other sulfonamides
Table 2A-2. Zone Diameter and MIC Breakpoints for *Salmonella* and *Shigella* spp.	**Revised:** • Comment regarding tetracycline susceptibility prediction for doxycycline and minocycline susceptibility
Table 2B-2. Zone Diameter and MIC Breakpoints for *Acinetobacter* spp.	**Added:** • Comment regarding minocycline for isolates that test intermediate by disk diffusion **Revised:** • Ampicillin-sulbactam disk diffusion breakpoints • Minocycline disk diffusion and MIC breakpoints ***Deleted:*** • Comment regarding tetracycline susceptibility prediction for doxycycline and minocycline • Doxycycline disk diffusion and MIC breakpoints • Tetracycline disk diffusion and MIC breakpoints

Overview of Changes (Continued)

Section/Table	Changes
Tables 2. (Continued)	
Table 2B-3. MIC Breakpoints for *Burkholderia cepacia* complex	**Added:** • Comment regarding removal of MIC breakpoints • Comment regarding ECVs • Comment regarding clinical reporting guidance • Comment regarding reference BMD as the only reproducible method ***Deleted:*** • Ceftazidime MIC breakpoints • Chloramphenicol MIC breakpoints • Levofloxacin MIC breakpoints • Meropenem MIC breakpoints • Minocycline MIC breakpoints • Ticarcillin-clavulanate MIC breakpoints • Trimethoprim-sulfamethoxazole MIC breakpoints
Table 2B-5. MIC Breakpoints for Other Non-Enterobacterales	**Revised:** • Comment regarding tetracycline susceptibility prediction for doxycycline and minocycline ***Deleted:*** • Comment regarding sulfisoxazole to represent other sulfonamides
Table 2C. Zone Diameter and MIC Breakpoints for *Staphylococcus* spp.	**Added:** • References describing species included in *Staphylococcus aureus* complex and the species evaluated by CLSI • List of methicillin (oxacillin) methods or targets appropriate for *Staphylococcus coagulans*; addition of *S. coagulans* to listing of species where breakpoints are applicable • Introduction of staphylococci other than *Staphylococcus aureus* (SOSA) terminology **Revised:** • Comment regarding resistance to the penicillinase-stable penicillins • Comment regarding tetracycline susceptibility prediction for doxycycline and minocycline • Comment regarding linezolid susceptibility prediction for tedizolid ***Deleted:*** • Comment regarding sulfisoxazole to represent other sulfonamides

Overview of Changes (Continued)

Section/Table	Changes
Tables 2. (Continued)	
Table 2D. Zone Diameter and MIC Breakpoints for *Enterococcus* spp.	**Revised:** • Comment regarding tetracycline susceptibility prediction for doxycycline and minocycline • Comment regarding linezolid susceptibility prediction for tedizolid
Table 2E. Zone Diameter and MIC Breakpoints for *Haemophilus influenzae* and *Haemophilus parainfluenzae*	**Revised:** • Comment regarding tetracycline susceptibility prediction for doxycycline and minocycline
Table 2F. Zone Diameter and MIC Breakpoints for *Neisseria gonorrhoeae*	**Revised:** • Comment regarding tetracycline susceptibility prediction for doxycycline and minocycline
Table 2G. Zone Diameter and MIC Breakpoints for *Streptococcus pneumoniae*	**Revised:** • Comment regarding tetracycline susceptibility prediction for doxycycline
Table 2H-1. Zone Diameter and MIC Breakpoints for *Streptococcus* spp. β-Hemolytic Group	**Revised:** • Comment regarding tetracycline susceptibility prediction for doxycycline and minocycline • Comment regarding linezolid susceptibility prediction for tedizolid
Table 2H-2. Zone Diameter and MIC Breakpoints for *Streptococcus* spp. Viridans Group	**Revised:** • Comment regarding tetracycline susceptibility prediction for doxycycline and minocycline • Comment regarding linezolid susceptibility prediction for tedizolid
Table 2I. Zone Diameter and MIC Breakpoints for *Neisseria meningitidis*	***Deleted:*** • Sulfisoxazole MIC breakpoints
Table 2J. MIC Breakpoints for Anaerobes	**Revised:** • Species appropriate for testing by broth microdilution (Testing Conditions box)
Table 2 Dosages. Antimicrobial Agent Dosage Regimens Used to Establish Susceptible or Susceptible-Dose Dependent Breakpoints	**Added:** • Dosage for ampicillin-sulbactam for *Acinetobacter* spp. • Dosage for minocycline for *Acinetobacter* spp. **Revised:** • Dosage for cefepime for *Pseudomonas aeruginosa*

Overview of Changes (Continued)

Section/Table	Changes
Tables 3. Specialized Resistance Testing	
Introduction to Tables 3B and 3C. Tests for Carbapenemases in Enterobacterales and *Pseudomonas aeruginosa*	**Added:** • Comment recommending testing for carbapenemase type for carbapenem-resistant Enterobacterales • Comment regarding false-negative eCIM results with isolates coproducing a serine carbapenemase and a metallo-β-lactamase
Table 3C. Modified Carbapenem Inactivation Methods for Suspected Carbapenemase Production in Enterobacterales and *Pseudomonas aeruginosa*	**Added:** • Comment regarding false-negative eCIM results with isolates coproducing a serine carbapenemase and a metallo-β-lactamase; comment includes reporting recommendations • Comment regarding poor sensitivity of eCIM for detection of metallo-β-lactamases in isolates coproducing a serine β-lactamase **Revised:** • QC recommendations box
Table 3D. Aztreonam Plus Ceftazidime-Avibactam Broth Disk Elution Method	**Added:** • Alternative QC strains
Table 3F-1. Test for Performing Disk Diffusion Directly From Positive Blood Culture Broth	**Added:** • Supplemental reading – options • Ranges for early reading (8–10 h) of select QC strain–antimicrobial agent combinations • Breakpoint additions since 2021 for: – Enterobacterales cefepime 8–10 h and 16–18 h – *P. aeruginosa* ceftazidime 8–10 h – *Acinetobacter* spp. ampicillin-sulbactam 8–10 h – *Acinetobacter* spp. ceftazidime 8–10 h – *Acinetobacter* spp. piperacillin-tazobactam 8–10 h and 16–18 h **Revised:** • Breakpoint revisions since 2021 for: – *Acinetobacter* spp. ampicillin-sulbactam 16–18 h
Table 3F-2. Zone Diameter Disk Diffusion Breakpoints for Enterobacterales Direct From Blood Culture	**Added:** • Breakpoints for cefepime 8–10 h and 16–18 h

Overview of Changes (Continued)

Section/Table	Changes
Tables 3. (Continued)	
Table 3F-3. Zone Diameter Disk Diffusion Breakpoints for *Pseudomonas aeruginosa* Direct From Blood Culture	**Added:** • Breakpoints for ceftazidime 8–10 h • Comment regarding intermediate results for ceftazidime
Table 3F-4. Zone Diameter Disk Diffusion Breakpoints for *Acinetobacter* spp. Direct From Blood Culture	**Added:** • Breakpoints for ampicillin-sulbactam 8–10 h • Breakpoints for ceftazidime 8–10 h • Breakpoints for piperacillin-tazobactam 8–10 h and 16–18 h **Revised:** • Breakpoints for ampicillin-sulbactam 16–18 h
Tables 4. Disk Diffusion QC Ranges and Associated Tables	
Table 4A-1. Disk Diffusion QC Ranges for Nonfastidious Organisms and Antimicrobial Agents Excluding β-Lactam Combination Agents	**Added:** • Footnote that sulfisoxazole can be used to represent any of the currently available sulfonamide preparations **Revised:** • Minocycline QC range for *Escherichia coli* ATCC®[a] 25922 • Footnote d regarding routine QC for erythromycin and clindamycin
Table 4A-2. Disk Diffusion QC Ranges for Nonfastidious Organisms and β-Lactam Combination Agents	**Added:** • Ceftibuten-avibactam QC ranges for: – *E. coli* ATCC® 25922 – *E. coli* NCTC 13353 – *Klebsiella pneumoniae* ATCC® 700603 – *K. pneumoniae* ATCC® BAA-1705™ – *K. pneumoniae* ATCC® BAA-2814™
Table 4C. Disk Diffusion Reference Guide to QC Frequency to Support Modifications to Antimicrobial Susceptibility Test Systems	**Revised:** • Title of table • Introduction regarding approaches to determine QC testing frequency following test modification ***Deleted:*** • Option for 15-replicate plan or 20- or 30-d plan

Overview of Changes (Continued)

Section/Table	Changes
Tables 5. MIC QC Ranges and Associated Tables	
Table 5A-1. MIC QC Ranges for Nonfastidious Organisms and Antimicrobial Agents Excluding β-Lactam Combination Agents	**Added:** • Zosurabalpin QC range for *Acinetobacter baumannii* NCTC 13304 • Footnote that sulfisoxazole can be used to represent any of the currently available sulfonamide preparations **Revised:** • Footnote o regarding exebacase testing instructions ***Deleted:*** • Detailed instructions and figures for testing exebacase (now in Appendix H2) • Sulfisoxazole QC instructions for CAMHB with 2.5–5% LHB in footnote h
Table 5A-2. MIC QC Ranges for Nonfastidious Organisms and β-Lactam Combination Agents	**Added:** • Ceftibuten-xeruborbactam QC ranges – *K. pneumoniae* ATCC® 700603 – *K. pneumoniae* ATCC® BAA-1705™ – *K. pneumoniae* ATCC® BAA-2814™
Table 5B. MIC QC Ranges for Fastidious Organisms (Broth Dilution Methods)	***Deleted:*** • Sulfisoxazole QC instructions for CAMHB with 2.5–5% LHB in footnote g
Table 5F. MIC Reference Guide to QC Frequency to Support Modifications to Antimicrobial Susceptibility Test Systems	**Revised:** • Title of table • Introduction regarding approaches to determine QC testing frequency following test modification ***Deleted:*** • Option for 15-replicate plan or 20- or 30-d plan
Tables 6. Preparing Antimicrobial Agent Stock Solutions	
Table 6A. Solvents and Diluents for Preparing Stock Solutions of Antimicrobial Agents	**Added:** • Zosurabalpin **Revised:** • Footnote i regarding exebacase handling instructions • Footnote j regarding CAMHB-HSD preparation instructions (now in Appendix H2)
Table 6C. Preparing Solutions and Media Containing Combinations of Antimicrobial Agents	**Added:** • Ceftibuten-xeruborbactam

Overview of Changes (Continued)

Section/Table	Changes
Appendixes	
Appendix A. Suggestions for Confirming Antimicrobial Susceptibility Test Results and Organism Identification for Agents Approved by the US Food and Drug Administration for Clinical Use	**Added:** • Sulbactam-durlobactam for *Acinetobacter baumanii* complex **Revised:** • Organization of organisms to align with organization of Tables 2
Appendix C. Quality Control Strains for Antimicrobial Susceptibility Tests	**Revised:** • NOTE regarding selection of QC strains for routine vs supplemental testing
Appendix F. Epidemiological Cutoff Values	**Added:** • *B. cepacia* complex ECVs for: – Ceftazidime – Levofloxacin – Meropenem – Minocycline – Trimethoprim-sulfamethoxazole **Revised:** • Order of the tables
Appendix H. Modifications of the Minimal Inhibitory Concentration Method for Testing Select Antimicrobial Agents (new)	**Added:** • Introductory text for Appendix H • Exebacase testing instructions in Appendix H, section H2 **Revised:** • Title for Appendix H
Appendix I. Selection of Quality Control Strains and Quality Control Testing Frequency (new)	New Appendix

Overview of Changes (Continued)

Section/Table	Changes
Glossaries	
Glossary I (Part 1). β-Lactams: Class and Subclass Designations and Generic Names	**Added:** • Ceftibuten-xeruborbactam
Glossary I (Part 2). Non–β-Lactams: Class and Subclass Designations and Generic Names	**Added:** • Zosurabalpin
Glossary II. Antimicrobial Agent Abbreviations, Routes of Administration, and Drug Class	**Added:** • Ceftibuten-xeruborbactam • Zosurabalpin

Abbreviations: AST, antimicrobial susceptibility testing; ATCC®, American Type Culture Collection; BMD, broth microdilution; CAMHB, cation-adjusted Mueller-Hinton broth; CAMHB-HSD, cation-adjusted Mueller-Hinton broth supplemented with horse serum (25% v/v) and 0.5 mM DL-dithiothreitol (pH 7.2–7.4); CSF, cerebrospinal fluid; d, day(s); eCIM, EDTA-modified carbapenem inactivation method; ECV, epidemiological cutoff value; EDTA, ethylenediaminetetraacetic acid; h, hour(s); **IQCP, individualized quality control plan;** LHB, lysed horse blood; MIC, minimal inhibitory concentration; NCTC, National Collection of Type Cultures; QC, quality control; **SOSA, staphylococci other than *Staphylococcus aureus*.**

Footnote

a. ATCC® is a registered trademark of the American Type Culture Collection.

CLSI Breakpoint Additions Since 2010

This table includes the CLSI M100 edition in which specific antimicrobial agent breakpoints were added for the first time for a specific organism group.

Antimicrobial Agent	Date of Addition (CLSI M100 edition)	Disk Diffusion Breakpoints	MIC Breakpoints	Comments
Enterobacterales				
Azithromycin	January 2015 (M100-S25)	X	X	*Salmonella enterica* ser. Typhi only
	March 2021 (M100-Ed31)	X	X	*Shigella* spp. Previously assigned an ECV
Cefiderocol	January 2019 (M100, 29th ed.)		X	
	January 2020 (M100, 30th ed.)	X		
Ceftaroline	January 2013 (M100-S23)	X	X	
Ceftazidime-avibactam	January 2018 (M100, 28th ed.)	X	X	
Ceftolozane-tazobactam	January 2016 (M100-S26)		X	
	January 2018 (M100, 28th ed.)	X		
Colistin	January 2020 (M100, 30th ed.)		X	Previously assigned an ECV
Doripenem	June 2010 (M100-S20-U)	X	X	
Imipenem-relebactam	March 2021 (M100-Ed31)	X	X	
Meropenem-vaborbactam	January 2019 (M100, 29th ed.)	X	X	
Pefloxacin	January 2015 (M100-S25)	X		*Salmonella* spp. (including *S. enterica* ser. Typhi) Surrogate test for ciprofloxacin
Plazomicin	March 2023 (M100-Ed33)	X	X	
Polymyxin B	January 2020 (M100, 30th ed.)		X	
Pseudomonas aeruginosa				
Cefiderocol	January 2019 (M100, 29th ed.)		X	
	January 2020 (M100, 30th ed.)	X		
Ceftazidime-avibactam	January 2018 (M100, 28th ed.)	X	X	
Doripenem	January 2012 (M100-S22)	X	X	
Imipenem-relebactam	March 2021 (M100-Ed31)	X	X	

CLSI Breakpoint Additions Since 2010 (Continued)

Antimicrobial Agent	Date of Addition (CLSI M100 edition)	Disk Diffusion Breakpoints	MIC Breakpoints	Comments
***Acinetobacter* spp.**				
Cefiderocol	January 2019 (M100, 29th ed.)		X	
	January 2020 (M100, 30th ed.)	X		
Doripenem	January 2014 (M100-S24)	X	X	
Sulbactam-durlobactam	February 2024 (M100-Ed34)	X	X	
Stenotrophomonas maltophilia				
Cefiderocol	January 2019 (M100, 29th ed.)		X	
	January 2020 (M100, 30th ed.)	X		
***Staphylococcus* spp.**				
Ceftaroline	January 2013 (M100-S23)	X	X	
Dalbavancin	January 2018 (M100, 28th ed.)		X	
Lefamulin	March 2021 (M100-Ed31)	X	X	
Oritavancin	January 2016 (M100-S26)		X	
Tedizolid	January 2016 (M100-S26)		X	*S. aureus* only
	February 2024 (M100-Ed34)	X		*S. aureus* only
Telavancin	January 2016 (M100-S26)	X	X	
***Enterococcus* spp.**				
Dalbavancin	January 2018 (M100, 28th ed.)		X	
Oritavancin	January 2016 (M100-S26)		X	
Tedizolid	January 2016 (M100-S26)		X	
Telavancin	January 2016 (M100-S26)	X	X	
Haemophilus influenzae* and *Haemophilus parainfluenzae				
Ceftaroline	January 2013 (M100-S23)	X	X	
Ceftolozane-tazobactam	March 2021 (M100-Ed31)		X	
Doripenem	January 2012 (M100-S22)	X	X	
Lefamulin	March 2021 (M100-Ed31)	X	X	
Neisseria gonorrhoeae				
Azithromycin	January 2019 (M100, 29th ed.)		X	Previously assigned an ECV
	March 2021 (M100-Ed31)	X		

CLSI Breakpoint Additions Since 2010 (Continued)

Antimicrobial Agent	Date of Addition (CLSI M100 edition)	Disk Diffusion Breakpoints	MIC Breakpoints	Comments
Streptococcus pneumoniae				
Ceftaroline	January 2013 (M100-S23)	X	X	
Doripenem	January 2012 (M100-S22)		X	
Doxycycline	January 2013 (M100-S23)	X	X	
Lefamulin	March 2021 (M100-Ed31)	X	X	
***Streptococcus* spp. β-Hemolytic Group**				
Ceftaroline	January 2013 (M100-S23)	X	X	
Dalbavancin	January 2018 (M100, 28th ed.)		X	
Doripenem	January 2012 (M100-S22)		X	
Oritavancin	January 2016 (M100-S26)		X	
Tedizolid	January 2016 (M100-S26)		X	
	February 2024 (M100-Ed34)	X		*S. pyogenes* and *S. agalactiae* only
Telavancin	January 2016 (M100-S26)	X	X	
***Streptococcus* spp. Viridans Group**				
Ceftolozane-tazobactam	January 2016 (M100-S26)		X	
Dalbavancin	January 2018 (M100, 28th ed.)		X	
Doripenem	January 2012 (M100-S22)		X	
Oritavancin	January 2016 (M100-S26)		X	
Tedizolid	January 2016 (M100-S26)		X	
	February 2024 (M100-Ed34)	X		*S. anginosus* group only
Telavancin	January 2016 (M100-S26)	X	X	
Anaerobes				
Doripenem	January 2012 (M100-S22)		X	
Imipenem-relebactam	March 2021 (M100-Ed31)		X	
Piperacillin-tazobactam	January 2017 (M100, 27th ed.)		X	
	January 2018 (M100, 28th ed.)		X	

Abbreviations: ECV, epidemiological cutoff value; MIC, minimal inhibitory concentration.

CLSI Breakpoint Revisions Since 2010

This table includes the CLSI M100 edition in which specific antimicrobial agent breakpoints were revised, updated, or deleted for a specific organism group. In some cases, unique breakpoints were added for a specific genus or species previously included within the organism or organism group breakpoints (eg, "*Salmonella* spp. [including *Salmonella enterica* ser. Typhi]" was previously grouped with the organism group breakpoints for Enterobacterales). Previous breakpoints for those revised here can be found in the edition of CLSI M100 that precedes the document listed in the column labeled "Date of Revision (CLSI M100 edition)." For example, previous breakpoints for aztreonam are listed in CLSI M100-S20 (January 2010). Deleted breakpoints can be found in CLSI Archived Resources.

Antimicrobial Agent	Date of Revision (CLSI M100 edition)	Disk Diffusion Breakpoints	MIC Breakpoints	Comments
Enterobacterales				
Amikacin	March 2023 (M100-Ed33)	X	X	
Aztreonam	January 2010 (M100-S20)	X	X	
Cefazolin (parenteral)	January 2010 (M100-S20)	X	X	Removed disk diffusion breakpoints
	January 2011 (M100-S21)	X	X	
	January 2016 (M100-S26)	X	X	For uncomplicated UTIs
Cefazolin (oral)	January 2014 (M100-S24)	X	X	Surrogate test for oral cephalosporins and uncomplicated UTIs
Cefepime	January 2014 (M100-S24)	X	X	Revised breakpoints include SDD
Cefiderocol	February 2022 (M100-Ed32)	X		
Cefotaxime	January 2010 (M100-S20)	X	X	
Ceftazidime	January 2010 (M100-S20)	X	X	
Ceftizoxime	January 2010 (M100-S20)	X	X	
Ceftolozane-tazobactam	February 2022 (M100-Ed32)	X		
Ceftriaxone	January 2010 (M100-S20)	X	X	
Ciprofloxacin	January 2012 (M100-S22)	X	X	*Salmonella* spp. (including *S. enterica* ser. Typhi)
	January 2019 (M100, 29th ed.)	X	X	Non-*Salmonella* spp.
Ertapenem	June 2010 (M100-S20-U)	X	X	
	January 2012 (M100-S22)	X	X	
Gentamicin	March 2023 (M100-Ed33)	X	X	
Imipenem	June 2010 (M100-S20-U)	X	X	
Levofloxacin	January 2013 (M100-S23)	X	X	*Salmonella* spp. (including *S. enterica* ser. Typhi)
	January 2019 (M100, 29th ed.)	X	X	Non-*Salmonella* spp.

CLSI Breakpoint Revisions Since 2010 (Continued)

Antimicrobial Agent	Date of Revision (CLSI M100 edition)	Disk Diffusion Breakpoints	MIC Breakpoints	Comments
Enterobacterales (Continued)				
Meropenem	June 2010 (M100-S20-U)	X	X	
Norfloxacin	January 2020 (M100, 30th ed.)	X	X	Reinstated breakpoints deleted from M100, 29th ed.
Ofloxacin	January 2013 (M100-S23)		X	*Salmonella* spp. (including *S. enterica* ser. Typhi)
Piperacillin	February 2022 (M100-Ed32)		X	Removed disk diffusion breakpoints due to reassessment of disk correlates for revised MIC breakpoints
Piperacillin-tazobactam	February 2022 (M100-Ed32)	X	X	
Tobramycin	March 2023 (M100-Ed33)	X	X	
Pseudomonas aeruginosa				
Amikacin	March 2023 (M100-Ed33)	X	X	Report only on organisms isolated from the urinary tract
Ciprofloxacin	January 2019 (M100, 29th ed.)	X	X	
Colistin	January 2017 (M100, 27th ed.)		X	
	January 2020 (M100, 30th ed.)		X	
Gentamicin	March 2023 (M100-Ed33)			Removed disk diffusion and MIC breakpoints
Imipenem	January 2012 (M100-S22)	X	X	
Levofloxacin	January 2019 (M100, 29th ed.)	X	X	
Meropenem	January 2012 (M100-S22)	X	X	
Norfloxacin	January 2020 (M100, 30th ed.)	X	X	Reinstated breakpoints deleted from M100, 29th ed.
Piperacillin	January 2012 (M100-S22)	X	X	
	March 2023 (M100-Ed33)	X	X	
Piperacillin-tazobactam	January 2012 (M100-S22)	X	X	
	March 2023 (M100-Ed33)	X	X	
Polymyxin B	January 2020 (M100, 30th ed.)		X	
Ticarcillin	January 2012 (M100-S22)	X	X	
Ticarcillin-clavulanate	January 2012 (M100-S22)	X	X	
Tobramycin	March 2023 (M100-Ed33)	X	X	

CLSI Breakpoint Revisions Since 2010 (Continued)

Antimicrobial Agent	Date of Revision (CLSI M100 edition)	Disk Diffusion Breakpoints	MIC Breakpoints	Comments
***Acinetobacter* spp.**				
Ampicillin-sulbactam	**January 2025 (M100-Ed35)**	**X**		
Cefiderocol	February 2022 (M100-Ed32)	X		
Colistin	January 2020 (M100, 30th ed.)		X	
Doxycycline	**January 2025 (M100-Ed35)**			**Removed disk diffusion and MIC breakpoints**
Imipenem	January 2014 (M100-S24)	X	X	
Meropenem	January 2014 (M100-S24)	X	X	
Minocycline	**January 2025 (M100-Ed35)**	**X**	**X**	
Polymyxin B	January 2020 (M100, 30th ed.)		X	
Tetracycline	**January 2025 (M100-Ed35)**			**Removed disk diffusion and MIC breakpoints**
***Burkholderia cepacia* complex**				
Ceftazidime	February 2024 (M100-Ed34)			Removed disk diffusion breakpoints
	January 2025 (M100-Ed35)			**Removed MIC breakpoints**
Chloramphenicol	**January 2025 (M100-Ed35)**			**Removed MIC breakpoints**
Levofloxacin	**January 2025 (M100-Ed35)**			**Removed MIC breakpoints**
Meropenem	February 2024 (M100-Ed34)			Removed disk diffusion breakpoints
	January 2025 (M100-Ed35)			**Removed MIC breakpoints**
Minocycline	February 2024 (M100-Ed34)			Removed disk diffusion breakpoints
	January 2025 (M100-Ed35)			**Removed MIC breakpoints**
Ticarcillin-clavulanate	**January 2025 (M100-Ed35)**			**Removed MIC breakpoints**
Trimethoprim-sulfamethoxazole	February 2024 (M100-Ed34)			Removed disk diffusion breakpoints
	January 2025 (M100-Ed35)			**Removed MIC breakpoints**
Stenotrophomonas maltophilia				
Cefiderocol	February 2022 (M100-Ed32)	X	X	
Ceftazidime	February 2024 (M100-Ed34)			Removed MIC breakpoints
Minocycline	February 2024 (M100-Ed34)	X	X	
Other Non-Enterobacterales				
Norfloxacin	January 2020 (M100, 30th ed.)	X	X	Reinstated breakpoints deleted from M100, 29th ed.

CLSI Breakpoint Revisions Since 2010 (Continued)

Antimicrobial Agent	Date of Revision (CLSI M100 edition)	Disk Diffusion Breakpoints	MIC Breakpoints	Comments
***Staphylococcus* spp.**				
Cefoxitin	January 2019 (M100, 29th ed.)	X		*S. epidermidis* surrogate test for oxacillin
Ceftaroline	January 2019 (M100, 29th ed.)	X	X	Revised breakpoints include SDD
Linezolid	February 2024 (M100-Ed34)	X		Staphylococci read with reflected light (previously read with transmitted light)
Norfloxacin	January 2020 (M100, 30th ed.)	X	X	Reinstated breakpoints deleted from M100, 29th ed.
Oxacillin	January 2016 (M100-S26)	X	X	*S. pseudintermedius*
	January 2018 (M100, 28th ed.)	X	X	*S. schleiferi*
	January 2019 (M100, 29th ed.)	X		*S. epidermidis*
	March 2021 (M100-Ed31)		X	*Staphylococcus* spp. except *S. aureus* and *S. lugdunensis*
Telavancin	January 2017 (M100, 27th ed.)			Removed disk diffusion breakpoints
***Enterococcus* spp.**				
Daptomycin	January 2019 (M100, 29th ed.)		X	
	January 2020 (M100, 30th ed.)		X	Separated into two sets of breakpoints: • *Enterococcus* spp. other than *E. faecium* • *E. faecium* (including SDD)
Norfloxacin	January 2020 (M100, 30th ed.)	X	X	Reinstated breakpoints deleted from M100, 29th ed.
Telavancin	January 2017 (M100, 27th ed.)			Removed disk diffusion breakpoints
Haemophilus influenzae* and *Haemophilus parainfluenzae				
Amoxicillin-clavulanate	February 2022 (M100-Ed32)		X	Removed disk diffusion breakpoints
Lefamulin	February 2022 (M100-Ed32)	X		For *H. influenzae* only
Streptococcus pneumoniae				
Lefamulin	February 2022 (M100-Ed32)	X		
Tetracycline	January 2013 (M100-S23)	X	X	
***Streptococcus* spp. β-Hemolytic Group**				
Telavancin	January 2017 (M100, 27th ed.)			Removed disk diffusion breakpoints
***Streptococcus* spp. Viridans Group**				
Telavancin	January 2017 (M100, 27th ed.)			Removed disk diffusion breakpoints
Neisseria meningitidis				
Sulfisoxazole	**January 2025 (M100, 35th ed.)**			**Removed MIC breakpoints**

Abbreviations: MIC, minimal inhibitory concentration; SDD, susceptible-dose dependent; UTI, urinary tract infection.

CLSI Archived Resources

The CLSI Archived Resources have been relocated to the CLSI website at www.clsi.org.

NOTE: The content of this document is supported by the CLSI consensus process and does not necessarily reflect the views of any single individual or organization.

Summary of CLSI Processes for Establishing Breakpoints and QC Ranges

The Clinical and Laboratory Standards Institute (CLSI) is an international, voluntary, not-for-profit, interdisciplinary, standards-developing, and educational organization accredited by the American National Standards Institute that develops and promotes the use of consensus-developed standards and guidelines within the health care community. These consensus standards and guidelines are developed in an open and consensus-seeking forum to cover critical areas of diagnostic testing and patient health care. CLSI is open to anyone or any organization that has an interest in diagnostic testing and patient care. Information about CLSI can be found at www.clsi.org.

The CLSI Subcommittee on Antimicrobial Susceptibility Testing reviews data from a variety of sources and studies (eg, *in vitro*, pharmacokinetics/pharmacodynamics, and clinical studies) to establish antimicrobial susceptibility test methods, breakpoints, and QC parameters. The details of the data necessary to establish breakpoints, QC parameters, and how the data are presented for evaluation are described in CLSI M23.[4]

Over time, a microorganism's susceptibility to an antimicrobial agent may decrease, resulting in a lack of clinical efficacy and/or safety. In addition, microbiological methods and QC parameters may be refined to ensure more accurate and better performance of susceptibility test methods. Because of these types of changes, CLSI continually monitors and updates information in its documents. Although CLSI standards and guidelines are developed using the most current information available at the time, the field of science and medicine is always changing; therefore, standards and guidelines should be used in conjunction with clinical judgment, current knowledge, and clinically relevant laboratory test results to guide patient treatment.

Additional information, updates, and changes in CLSI M100 are found in the meeting summary minutes of the CLSI Subcommittee on Antimicrobial Susceptibility Testing at https://clsi.org/meetings/ast-file-resources/.

CLSI Methods vs Commercial Methods and CLSI vs US Food and Drug Administration Breakpoints

The standard methods described in CLSI M07[2] and CLSI M100 are reference methods. These methods, **and the standard disk diffusion method described in CLSI M02,[1]** may be used for routine antimicrobial susceptibility testing of patient isolates, for evaluating commercial devices that will be used in medical laboratories, by drug or device manufacturers for testing new agents or systems, or for surveillance of antimicrobial resistance. Results generated by reference methods, such as those included in CLSI documents, may be used by regulatory authorities to evaluate the performance of commercial susceptibility testing devices as part of the approval process. Clearance by a regulatory authority indicates the commercial susceptibility testing device provides susceptibility results that are substantially equivalent to results generated using reference methods for the organisms and antimicrobial agents described in the device manufacturer's approved package insert.

CLSI breakpoints may differ from those approved by various regulatory authorities for many reasons, including use of different databases, differences in data interpretation, differences in doses used in different parts of the world, and public health policies. Differences also exist because CLSI proactively evaluates the need for changing breakpoints. The reasons why breakpoints may change and the manner in which CLSI evaluates data and determines breakpoints are outlined in CLSI M23.[4]

Following a decision by CLSI to change an existing breakpoint, regulatory authorities may also review data to determine how changing breakpoints may affect the safety and effectiveness of the antimicrobial agent for the approved indications. If the regulatory authority changes breakpoints, commercial device manufacturers may have to conduct a clinical trial, submit the data to the regulatory authority, and await review and approval. For these reasons, a delay of one or more years may be needed if a breakpoint and interpretive category change is to be implemented by a device manufacturer. In the United States, it is acceptable for laboratories that use US Food and Drug Administration (FDA)–cleared susceptibility testing devices to use existing FDA breakpoints. Either FDA or CLSI susceptibility breakpoints are acceptable to laboratory accrediting organizations in the United States. Policies in other countries may vary. Each laboratory should check with the manufacturer of its antimicrobial susceptibility test system for additional information on the breakpoints and interpretive categories used in its system's software.

CLSI Subcommittee on Antimicrobial Susceptibility Testing Mission Statement

The CLSI Subcommittee on Antimicrobial Susceptibility Testing is composed of representatives from the professions, government, and industry, including microbiology laboratories, government agencies, health care providers and educators, and pharmaceutical and diagnostic microbiology industries. Using the CLSI voluntary consensus process, the subcommittee develops standards that promote accurate antimicrobial susceptibility testing and appropriate reporting. The mission of the CLSI Subcommittee on Antimicrobial Susceptibility Testing is to:

- Develop standard reference methods for antimicrobial susceptibility tests.
- Provide QC parameters for standard test methods.
- Establish breakpoints and interpretive categories for the results of standard antimicrobial susceptibility tests and provide epidemiological cutoff values when breakpoints are not available.
- Provide suggestions for testing and reporting strategies that are clinically relevant and cost-effective.
- Continually refine standards and optimize detection of emerging resistance mechanisms through development of new or revised methods, breakpoints, and QC parameters.
- Educate users through multimedia communication of standards and guidelines.
- Foster a dialogue with users of these methods and those who apply them.

The ultimate purpose of the subcommittee's mission is to provide useful information to enable laboratories to assist the clinician in the selection of appropriate antimicrobial therapy for patient care. The standards and guidelines are meant to be comprehensive and to include all antimicrobial agents for which the data meet established CLSI guidelines. The values that guide this mission are quality, accuracy, fairness, timeliness, teamwork, consensus, and trust.

Instructions for Use of Tables

These instructions apply to:

- Tables 1A through 1J: suggested tiers of antimicrobial agents that should be considered for testing and reporting by microbiology laboratories. These suggestions include clinical efficacy, current consensus recommendations for first-choice and alternative drugs, and US Food and Drug Administration (FDA) clinical indications for use. In other countries, placement of antimicrobial agents in Tables 1A through 1J should be based on available drugs approved for clinical use by relevant regulatory organizations.
- Tables 2A through 2J: tables for each organism group that contain:
  - Recommended testing conditions
  - Routine QC recommendations (also see CLSI M02[1] and CLSI M07[2])
  - General comments for testing the organism group and specific comments for testing particular agent/organism combinations
  - Agents that should be considered for routine testing and reporting by medical microbiology laboratories, as specified in Tables 1A through 1J (test/report Tiers 1, 2, 3, and 4), including agents reported only on organisms isolated from the urinary tract (designated by "U")
  - Agents that are appropriate for the respective organism group but are not listed in Tables 1 and would generally not warrant routine testing by a medical microbiology laboratory in the United States (designated with an asterisk as "other"; designated with "Inv." for "investigational" [not yet FDA approved]), including agents reported only on organisms isolated from the urinary tract (designated by "U")
  - Zone diameter and minimal inhibitory concentration (MIC) breakpoints
- Tables 1J and 2J: tables containing specific recommendations for testing and reporting results on anaerobes and some of the information listed in the bullets above
- Tables 3A through 3L: tables describing tests to detect particular resistance types in specific organisms or organism groups

I. Selecting Antimicrobial Agents for Testing and Reporting

A. Appropriate Agents for Routine Testing

> Selecting the most appropriate antimicrobial agents to test and report is a decision best made by each laboratory in consultation with the antimicrobial stewardship team and other relevant institutional stakeholders.

The suggestions for each organism group in Tables 1A through 1J include agents of proven efficacy that show acceptable *in vitro* test performance. Considerations in the assignment of agents to specific tiers include:

- Clinical efficacy
- Prevalence of resistance
- Minimizing emergence of resistance
- FDA clinical indications for use
- Current consensus recommendations for first-choice and alternative drugs
- Cost

Tests on selected agents may be useful for infection-prevention purposes (eg, testing ceftazidime for Enterobacterales to indicate potential extended-spectrum β-lactamase production; see Table 3A).

B. Equivalent Agents

Antimicrobial agents listed together in a single box are agents for which interpretive categories (susceptible, intermediate, susceptible-dose dependent, or resistant) and clinical efficacy are similar. A laboratory will often test only one agent from a box routinely, typically the agent that is on its formulary. In some cases, a laboratory may not test any agents from a box, depending on institutional needs.

In some boxes, the agents will be listed with an "or" between them. The "or" identifies agents for which cross-resistance and cross-susceptibility are nearly complete. Results from one agent connected by an "or" can be used to predict results for the other agent (ie, equivalent agents). For example, Enterobacterales susceptible to cefotaxime can be considered susceptible to ceftriaxone. The results obtained from testing cefotaxime could be reported along with a comment that the isolate is also susceptible to ceftriaxone. For drugs connected with an "or," combined major and very major errors are fewer than 3%, and minor errors are fewer than 10%, based on a large population of bacteria tested (see CLSI M23[4] for description of error types). In addition, to qualify for an "or," at least 100 strains with resistance to the agents in question must be tested and a result of "resistant" must be obtained with all agents for at least 95% of the strains. "Or" is also used for comparable agents when tested against organisms for which "susceptible-only" breakpoints are provided (eg, cefotaxime or ceftriaxone with *Haemophilus influenzae*). When no "or" connects agents within a box, testing of one agent cannot be used to predict results for another, owing either to discrepancies or insufficient data (see Section VIII, which describes equivalent agent tests).

C. Test/Report Tiers and Additional Designations

Antimicrobial Agent Test and Report Tiers and Additional Considerations for Agents Listed in Tables 1

Tier	Definition	Test	Report[a]	Additional Testing and Reporting Considerations
1	Antimicrobial agents that are appropriate for routine, primary testing and reporting	Routine	Routine	
2	Antimicrobial agents that are appropriate for routine, primary testing but may be reported following cascade reporting rules established at each institution	Routine	Cascade	• Report following cascade reporting rules due to resistance to agent(s) in Tier 1. • May be reported routinely based on institution-specific guidelines.
3	Antimicrobial agents that are appropriate for routine, primary testing in institutions that serve patients at high risk for MDROs but should only be reported following cascade reporting rules established at each institution[b]	Routine or by request	Cascade	Test routinely based on institution-specific guidelines or by clinician request and report following cascade reporting rules due to resistance to agent(s) in Tiers 1 and 2.
4	Antimicrobial agents that may warrant testing and reporting by clinician request if antimicrobial agents in other tiers are not optimal because of various factors	By request	By request	• Test and report by clinician request due to: – Unavailability of preferred drug for clinical use – Patient underlying condition, including allergies – Unusual susceptibility profile of the organism, including resistance to agents in Tiers 1, 2, and 3 – Polymicrobial infection • May also warrant testing as an epidemiological aid (eg, testing ceftazidime for Enterobacterales to indicate potential ESBL production; see Table 3A).
Urine only	Antimicrobial agents designated by a "(U)" in Tables 2 should be reported only on organisms isolated from the urinary tract.	Routine	Report as appropriate	Agents in Tiers 1, 2, and 3 may also be reported on urine isolates, as appropriate, following the testing and reporting guidance for the respective tiers.

Abbreviations: ESBL, extended-spectrum β-lactamase; MDRO, multidrug-resistant organism; UTI, urinary tract infection.

Footnotes

a. Antimicrobial agents should be reported selectively, as appropriate (eg, because it is effective in treating uncomplicated UTIs only, nitrofurantoin would be reported only on isolates from urine). Refer to section D for definition of cascade reporting.

b. Identification of patients at high risk for MDROs will likely be communicated by infection preventionists. For examples of criteria used to identify patients at high risk for MDROs, see https://www.cdc.gov/mrsa/about/index.html and https://www.cdc.gov/esbl-producing-enterobacterales/about/index.html

Antimicrobial Agent Test and Report Designations and Additional Considerations for Agents Not Listed in Tables 1

Designation	Definition	Test	Report[a]	Additional Testing and Reporting Considerations
Other	Antimicrobial agents with established clinical breakpoints designated by an * in Tables 2 that are generally not candidates for testing and reporting in the United States	By request	By request	• Test and report only by clinician request and only following consultation with the antimicrobial stewardship team and other relevant institutional stakeholders to ensure appropriateness of the request. • Agents with an “Other” designation may not reflect current consensus recommendations for first-choice and alternative drugs for the specific organism or organism group.
Inv.	Antimicrobial agents that are investigational for the organism group designated by “Inv.” in Tables 2 have not yet been approved by the FDA for use in the United States.	By request	By request	Test and report only by clinician request and only following consultation with the antimicrobial stewardship team and other relevant institutional stakeholders to ensure appropriateness of the request. These agents would likely be clinically available for compassionate use only.

Abbreviations: FDA, US Food and Drug Administration; UTI, urinary tract infection.

Footnote

a. Antimicrobial agents should be reported selectively, as appropriate (eg, because it is effective in treating uncomplicated UTIs only, nitrofurantoin would be reported only on isolates from urine).

D. Selective and Cascade Reporting

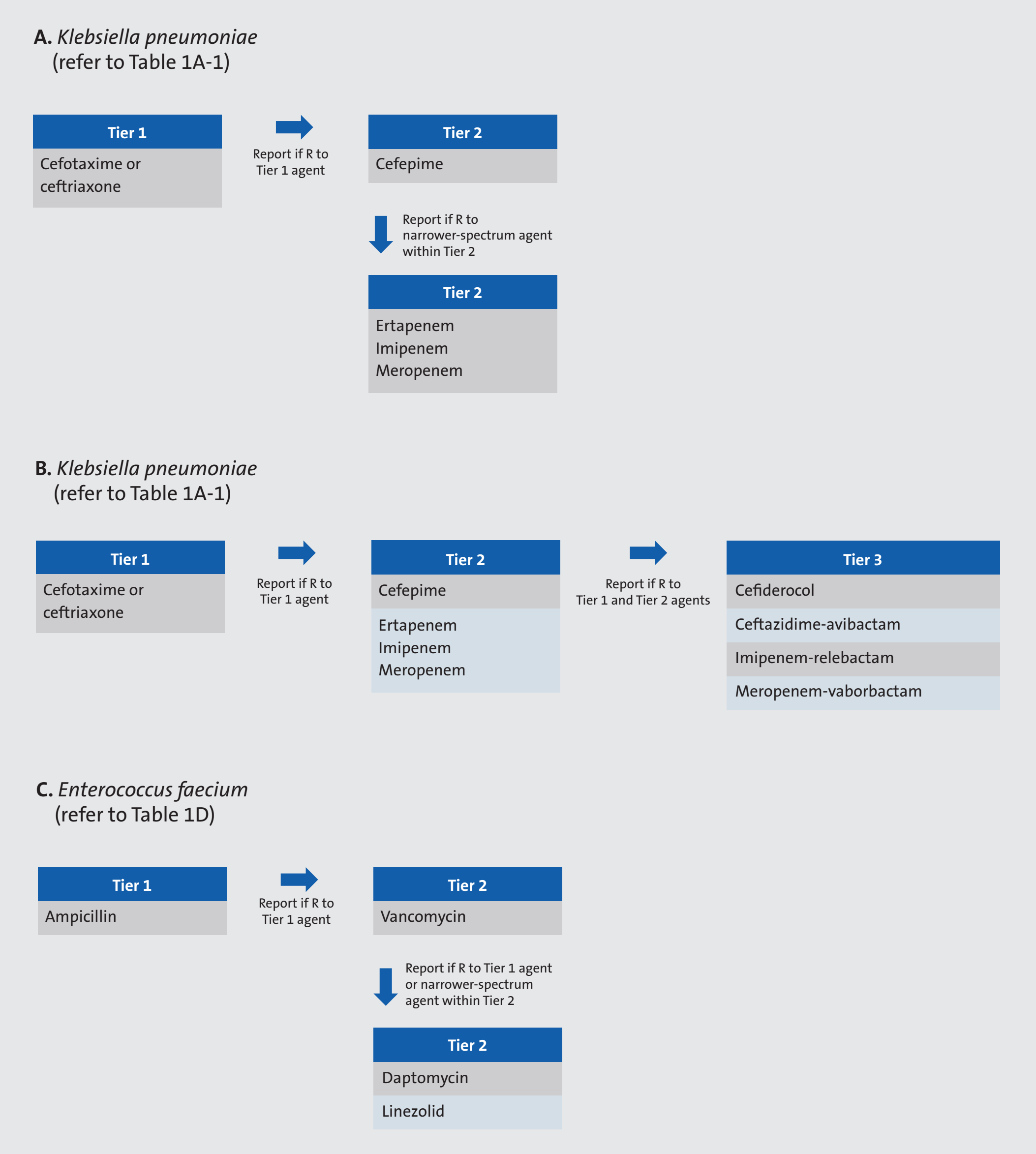
Each laboratory should consider developing selective and/or cascade reporting rules in consultation with the antimicrobial stewardship team and other relevant institutional stakeholders. Selective and cascade reporting is done to encourage appropriate antimicrobial agent use. The positioning of drugs in Tables 1A through 1J can be used to guide development of selective and/or cascade reporting rules.

Selective reporting involves reporting results for specific antimicrobial agents based on defined criteria unrelated to results obtained from antimicrobial susceptibility testing (AST) (eg, organism identification, body site, clinical setting, or patient demographics). For example, nitrofurantoin would be reported only on isolates from urine because it is effective in treating uncomplicated urinary tract infections only. Daptomycin is not reported for isolates recovered from the lower respiratory tract because it interacts with pulmonary surfactant, resulting in inhibition of antibacterial activity. First- and second-generation cephalosporins are not reported on *Salmonella* spp. because of their ineffectiveness in treating patients with *Salmonella* infections.

Cascade reporting involves reporting results for specific agents based on the overall antimicrobial susceptibility profile of an isolate. Results for secondary or broader-spectrum agents (eg, Tier 2 or 3) are reported only if the isolate is resistant to primary or narrower-spectrum agents (eg, Tier 1). For example, if a *Klebsiella pneumoniae* isolate is resistant to ceftriaxone, cefepime might be reported. However, cefepime might be suppressed in a ceftriaxone-susceptible *K. pneumoniae* isolate. A "resistant" result for a broader-spectrum agent (eg, Tier 2) should always be reported even if the organism tests "susceptible" to the narrower-spectrum agent (eg, Tier 1). Such unexpected resistant results should be confirmed (see Appendix A, footnote a).

Cascade rules can be created for agents within the same tier or between tiers. Agents listed in the same row between tiers in Tables 1A through 1J can be used as a guide for creating cascade reporting rules. For example, if a *K. pneumoniae* isolate is ceftriaxone resistant (Tier 1), cascade reporting can be initiated for cefepime and/or carbapenems (Tier 2). If the *K. pneumoniae* isolate is resistant to ceftriaxone, cefepime, and a carbapenem, cascade reporting of cefiderocol, ceftazidime-avibactam, imipenem-relebactam, and/or meropenem-vaborbactam (Tier 3) may be considered (see Figure 1, examples A and B). If an *Enterococcus faecium* isolate is ampicillin resistant (Tier 1) and vancomycin resistant (Tier 2), cascade reporting of daptomycin and linezolid (Tier 2) may be considered (see Figure 1, example C).

Each laboratory should develop a protocol to test additional agents on isolates that are confirmed as resistant to all agents on their routine test panels. This protocol should include options for testing additional agents in-house or sending the isolate to a referral laboratory.


A. *Klebsiella pneumoniae*
(refer to Table 1A-1)
Tier 1
Cefotaxime or ceftriaxone
Report if R to Tier 1 agent
Tier 2
Cefepime
Report if R to narrower-spectrum agent within Tier 2
Tier 2
Ertapenem
Imipenem
Meropenem
B. *Klebsiella pneumoniae*
(refer to Table 1A-1)
Tier 1
Cefotaxime or ceftriaxone
Report if R to Tier 1 agent
Tier 2
Cefepime
Ertapenem
Imipenem
Meropenem
Report if R to Tier 1 and Tier 2 agents
Tier 3
Cefiderocol
Ceftazidime-avibactam
Imipenem-relebactam
Meropenem-vaborbactam
C. *Enterococcus faecium*
(refer to Table 1D)
Tier 1
Ampicillin
Report if R to Tier 1 agent
Tier 2
Vancomycin
Report if R to Tier 1 agent or narrower-spectrum agent within Tier 2
Tier 2
Daptomycin
Linezolid

Abbreviation: R, resistant.

Figure 1. Cascade Reporting Examples. Cascade reporting within tiers (A, C) and between tiers (A, B, and C).

II. Breakpoint and Interpretive Category Definitions

A. Breakpoint Definition

breakpoint – minimal inhibitory concentration (MIC) or zone diameter value used to categorize an organism as susceptible, susceptible-dose dependent, intermediate, resistant, or nonsusceptible; **NOTE 1:** MIC or zone diameter values generated by a susceptibility test can be interpreted based on established breakpoints; **NOTE 2:** Because breakpoints are largely based on pharmacologically and clinically rich datasets using *in vitro* and *in vivo* data, they are considered robust predictors of likely clinical outcomes; **NOTE 3:** Also known as "clinical breakpoint"; **NOTE 4:** See **interpretive category.**

B. Interpretive Category Definition

interpretive category – category derived from microbiological characteristics, pharmacokinetic/pharmacodynamic parameters, and clinical outcome data, when available; **NOTE 1:** minimal inhibitory concentration or zone diameter values generated by a susceptibility test can be interpreted based on established breakpoints; **NOTE 2:** See **breakpoint.**

EXAMPLE:

Interpretive Category	Breakpoints	
	MIC, µg/mL	Zone Diameter, mm
Susceptible	≤ 4	≥ 20
Susceptible-dose dependent	8–16	15–19
Intermediate	8–16	15–19
Resistant	≥ 32	≤ 14
Nonsusceptible	> 1	< 17

MIC or zone diameter value breakpoints and interpretive categories are established per CLSI M23[4] for categories of susceptible, intermediate, and resistant (and susceptible-dose dependent and nonsusceptible, when appropriate). CLSI susceptible (S) or susceptible-dose dependent (SDD) breakpoints added or revised since 2010 have been based on specific dosage regimen(s); these dosage regimens are listed in "Table 2 Dosages. Antimicrobial Agent Dosage Regimens Used to Establish Susceptible or Susceptible-Dose Dependent Breakpoints" (referred to as "Table 2 Dosages").

- **susceptible (S)** – category defined by a breakpoint that implies that isolates with an MIC at or below or a zone diameter at or above the susceptible breakpoint are inhibited by the usually achievable concentrations of antimicrobial agent when the dosage recommended to treat the site of infection is used, resulting in likely clinical efficacy.

- **susceptible-dose dependent (SDD)** – a category defined by a breakpoint that implies that susceptibility of an isolate depends on the dosage regimen that is used in the patient. To achieve levels that are likely to be clinically effective against isolates for which the susceptibility testing results (either MICs or zone diameters) are in the SDD category, it is necessary to use a dosage regimen (ie, higher doses, more frequent doses, or both, or extended infusion) that results in higher drug exposure than that achieved with the dose that was used to establish the susceptible breakpoint. Consideration should be given to the maximum, literature-supported dosage regimen because higher exposure gives the highest probability of adequate coverage of an SDD isolate. Table 2 Dosages lists the doses used when establishing SDD categories. The drug label should be consulted for recommended doses and adjustment for organ function; **NOTE:** The SDD category may be assigned when doses well above those used to calculate the susceptible breakpoint are supported by the literature, widely used clinically, and/or approved and for which sufficient data to justify the designation exist and have been reviewed. This category also includes a buffer zone for inherent variability in test methods, which should prevent small, uncontrolled, technical factors from causing major discrepancies in interpretations, especially for drugs with narrow pharmacotoxicity margins. See Appendix E for additional information.
- **intermediate (I)** – a category defined by a breakpoint that includes isolates with MICs or zone diameters within the intermediate range that approach usually attainable blood and tissue levels and/or for which response rates may be lower than for susceptible isolates; **NOTE:** An I with a ^ in Tables 2 indicates agents that have the potential to concentrate in the urine. The I^ is for informational use only. The decision to report I^ is best made by each laboratory based on institution-specific guidelines and in consultation with appropriate medical personnel. The I category also includes a buffer zone for inherent variability in test methods, which should prevent small, uncontrolled, technical factors from causing major discrepancies in interpretations, especially for drugs with narrow pharmacotoxicity margins.
- **resistant (R)** – a category defined by a breakpoint that implies that isolates with an MIC at or above or a zone diameter at or below the resistant breakpoint are not inhibited by the usually achievable concentrations of the agent with normal dosage schedules and/or that demonstrate MICs or zone diameters that fall in the range in which specific microbial resistance mechanisms are likely, and clinical efficacy of the agent against the isolate has not been reliably shown in treatment studies.
- **nonsusceptible (NS)** – a category used for isolates for which only a susceptible breakpoint is designated because of the absence or rare occurrence of resistant strains. Isolates for which the antimicrobial agent MICs are above or the zone diameters are below the value indicated for the susceptible breakpoint should be reported as nonsusceptible; **NOTE 1:** An isolate that is interpreted as nonsusceptible does not necessarily mean that the isolate has a resistance mechanism. It is possible that isolates with MICs above the susceptible breakpoint that lack resistance mechanisms may be encountered within the wild-type distribution after the time the susceptible-only breakpoint was set; **NOTE 2:** The term "nonsusceptible" should not be used when the text is describing an organism-drug category with intermediate and resistant interpretive categories. Isolates that are in the categories of "intermediate" or "resistant" could be called "not susceptible" rather than "nonsusceptible."

C. Example of Breakpoints and Interpretive Categories as Used in Tables 2

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, Nearest Whole mm			Interpretive Categories and MIC Breakpoints, µg/mL		
		S	I[a]	R	S	I[a]	R
X	30 µg	≥ 20	15–19	≤ 14	≤ 4	8–16	≥ 32
Y	–	–	–	–	≤ 1	2	≥ 4
Z	10 µg	≥ 16	–	–	≤ 1	–	–

Abbreviations: I, intermediate; MIC, minimal inhibitory concentration; R, resistant; S, susceptible; SDD, susceptible-dose dependent.

[a] Or SDD, if appropriate.

For antimicrobial agent X with breakpoints in the table above, the susceptible breakpoint is ≤ 4 µg/mL or ≥ 20 mm and the resistant breakpoint is ≥ 32 µg/mL or ≤ 14 mm. For some antimicrobial agents (eg, antimicrobial agent Y), only MIC breakpoints may be available. For these agents, the disk diffusion zone diameters do not correlate with MIC values or data have not been evaluated as described in CLSI M23.[4] Technical issues may also preclude the use of the disk diffusion method for some agents. For some antimicrobial agents (eg, antimicrobial agent Z) only a "susceptible" category exists. For these agents, the absence or rare occurrence of resistant strains precludes defining any results categories other than "susceptible." For strains yielding results suggestive of a "nonsusceptible" category, organism identification and antimicrobial susceptibility test results should be confirmed (see Appendix A). In examples Y and Z, a dash mark (–) indicates a disk is not available or that breakpoints are not applicable.

III. Reporting Results

A. Organisms Included in Tables 2

The MIC values determined as described in CLSI M07[2] may be reported directly to clinicians for patient care purposes. However, it is essential that an interpretive category result (S, SDD, I, R, or NS) also be provided routinely to facilitate understanding of the MIC report by clinicians. Zone diameter measurements without an interpretive category should not be reported. Recommended interpretive categories for various MIC and zone diameter values are included in tables for each organism group and are based on the evaluation of data as described in CLSI M23.[4]

Laboratories should report results only for agents listed in Tables 2 specific to the organism being tested. It is not appropriate to apply disk diffusion or MIC breakpoints borrowed from a table in which the organism is not listed. There may be rare cases for which an agent may be appropriate for an isolate but for which there are no CLSI breakpoints (eg, tigecycline). In these cases, the FDA Susceptibility Test Interpretive Criteria website (https://www.fda.gov/drugs/development-resources/fda-recognized-antimicrobial-susceptibility-test-interpretive-criteria) and the prescribing information document for the agent should be consulted.

For more information on reporting epidemiological cutoff values in the medical laboratory, see Appendix F.

B. Organisms Excluded From Tables 2

For some organism groups excluded from Tables 2A through 2J, CLSI M45[5] provides suggestions for standardized methods for AST, including information about drug selection, interpretation, and QC. The organism groups covered in that guideline are *Abiotrophia* and *Granulicatella* spp. (formerly known as nutritionally deficient or nutritionally variant streptococci); *Aerococcus* spp.; *Aeromonas* spp. (including members of *Aeromonas caviae* complex, *Aeromonas hydrophila* complex, and *Aeromonas veronii* complex); *Bacillus* spp. (not *Bacillus anthracis*); *Campylobacter jejuni/coli*; *Corynebacterium* spp. (including *Corynebacterium diphtheriae*); *Erysipelothrix rhusiopathiae*; *Gemella* spp.; the HACEK group: *Aggregatibacter* spp. (formerly *Haemophilus aphrophilus*, *Haemophilus paraphrophilus*, *Haemophilus segnis*, and *Actinobacillus actinomycetemcomitans*), *Cardiobacterium* spp., *Eikenella corrodens*, and *Kingella* spp.; *Helicobacter pylori*; *Lactobacillus* spp.; *Lactococcus* spp.; *Leuconostoc* spp.; *Listeria monocytogenes*; *Micrococcus* spp.; *Moraxella catarrhalis*; *Pasteurella* spp.; *Pediococcus* spp.; *Rothia mucilaginosa*; potential agents of bioterrorism; and *Vibrio* spp. (including *Vibrio cholerae*).

For organisms other than those in the groups mentioned above, studies are not yet adequate to develop reproducible, definitive standards to interpret results. These organisms may need different media or different incubation atmospheres, or they may show marked strain-to-strain variation in growth rate and should not be tested routinely. For these microorganisms, consultation with an infectious diseases specialist is recommended for guidance in determining the need for susceptibility testing and in results interpretation. Published reports in the medical literature and current consensus recommendations for therapy of uncommon microorganisms may preclude the need for testing. If necessary, a dilution method usually is the most appropriate testing method, and this may necessitate submitting the organism to a referral laboratory. Physicians should be informed of the limitations of results and advised to interpret results with caution.

C. Cumulative Antibiograms

Policies regarding the generation of cumulative antibiograms should be developed together with the antimicrobial stewardship team and other relevant institutional stakeholders. See CLSI M39[6] for detailed instructions on generating cumulative antibiograms.

D. Minimal Inhibitory Concentration Reporting Concentrations

When serial twofold dilution MICs are being prepared and tested, the actual dilution scheme is, eg:

16, 8, 4, 2, 1, 0.5, 0.25, 0.125, 0.0625, 0.03125 µg/mL, etc. (see Table 7 for additional dilutions).

For convenience only, not because these are the actual concentrations tested, it was decided to use the following values in CLSI M100: 16, 8, 4, 2, 1, 0.5, 0.25, 0.12, 0.06, 0.03 µg/mL, etc.

The values that appear in the tables are equivalent to the actual values tested, eg, 0.12 µg/mL = 0.125 µg/mL, and laboratories should report an MIC of ≤ 0.125 µg/mL as ≤ 0.12 µg/mL.

IV. Therapy-Related Comments and Dosage Regimens

Some comments in the tables relate to therapy concerns. These are denoted with an ***Rx*** symbol. It may be appropriate to include some of these comments (or modifications thereof) on the patient report. An example would be inclusion of a comment when rifampin is being reported stating that "Rifampin should not be used alone for antimicrobial therapy." Antimicrobial dosage regimens often vary widely among specialists and institutions. In some cases, the MIC breakpoints rely on pharmacokinetic/pharmacodynamic data, using specific human dosage regimens. For breakpoints for newer agents or for breakpoints that have been recently reevaluated, when specific dosage regimens are important for properly applying breakpoints, the dosage regimen is listed in the Dosage Regimens Used to Establish Susceptible or Susceptible-Dose Dependent Breakpoints table. These dosage regimen comments are not generally intended for use on individual patient reports.

V. Confirmation of Patient Results

Multiple test parameters are monitored by following the QC recommendations described in CLSI M100. However, acceptable results derived from testing QC strains do not guarantee accurate results when testing patient isolates. It is important to review all the results obtained from all drugs tested on a patient's isolate before reporting the results. This review should include but not be limited to ensuring that (1) the AST results are consistent with the identification of the isolate; (2) the results from individual agents within a specific drug class follow the established hierarchy of activity rules (eg, in general, third-generation cephems are more active than first- or second-generation cephems against Enterobacterales); and (3) the isolate is susceptible to those agents for which resistance has not been documented (eg, vancomycin and *Streptococcus* spp.) and for which only "susceptible" breakpoints exist in CLSI M100.

Unusual or inconsistent results should be confirmed by rechecking various testing parameters detailed in Appendix A. Each laboratory must develop its own policies for confirming unusual or inconsistent antimicrobial susceptibility test results. The list provided in Appendix A emphasizes results that are most likely to affect patient care.

VI. Development of Resistance and Testing of Repeat Isolates

Isolates that are initially susceptible may become intermediate or resistant after therapy is initiated. Therefore, subsequent isolates of the same species from a similar anatomical site should be tested to detect resistance that may have developed. Development of resistance can occur within a few days after initiation of therapy and has been noted most frequently in *Citrobacter freundii* complex, *Enterobacter cloacae* complex, and *Klebsiella* (formerly *Enterobacter*) *aerogenes* with third-generation cephalosporins; in *Pseudomonas aeruginosa* with all antimicrobial agents; and in staphylococci with fluoroquinolones. For *Staphylococcus aureus*, vancomycin-susceptible isolates may become vancomycin intermediate during the course of prolonged therapy.

In certain circumstances, the decision to perform susceptibility tests on subsequent isolates necessitates knowledge of the specific situation and the severity of the patient's condition, eg, an isolate of *E. cloacae* complex from a blood culture on a premature infant or methicillin (oxacillin)-resistant *Staphylococcus aureus* (MRSA) from a patient with prolonged bacteremia. Laboratory guidelines on when to perform susceptibility testing on repeat isolates should be determined after consultation with the medical staff.

VII. Warning

Some of the comments in the tables relate to dangerously misleading results that can occur when certain antimicrobial agents are tested and reported as susceptible against specific organisms. These are denoted with the word **"Warning."**

Locations	Organisms	Antimicrobial Agents
"Warning": The following antimicrobial agent–organism combinations may appear active *in vitro* but are not effective clinically and must not be reported as susceptible.		
Table 2A-2	*Salmonella* spp., *Shigella* spp.	First- and second-generation cephalosporins, cephamycins, and aminoglycosides
Table 2D	*Enterococcus* spp.	Aminoglycosides (except for high-level resistance testing), cephalosporins, clindamycin, and trimethoprim-sulfamethoxazole
"Warning": Do not report the following antimicrobial agents for bacteria isolated from CSF. These are not the drugs of choice and may not be effective for treating CSF infections caused by the bacteria included in Tables 2A–2J:		
Tables 2A–2J	Bacteria isolated from CSF	Agents administered by oral route only, first- and second-generation cephalosporins and cephamycins, doripenem, ertapenem, imipenem, clindamycin, lefamulin, macrolides, and tetracyclines.

Abbreviation: CSF, cerebrospinal fluid.

VIII. Routine, Supplemental, Screening, Surrogate Agent, and Equivalent Agent Testing to Determine Susceptibility and Resistance to Antimicrobial Agents

The testing categories are defined as follows:

- **Routine test:** disk diffusion or broth or agar dilution MIC tests for routine clinical testing
- **Supplemental (not routine) test:** test that detects susceptibility or resistance to a drug or drug class by method other than routine disk diffusion or broth or agar dilution MIC and does not need additional tests to confirm susceptibility or resistance
  - Some supplemental tests identify a specific resistance mechanism and may be required or optional for reporting specific clinical results.
- **Screening test:** test that provides presumptive results; additional testing typically only needed for a specific result (eg, only if screen is positive)
- **Surrogate agent test:** test performed with an agent that replaces a test performed with the antimicrobial agent of interest and is used when the agent of interest cannot be tested due to unavailability of the agent or performance issues (eg, surrogate agent performs better than the agent of interest)

- **Equivalent agent test:** test performed with an agent that predicts results of closely related agents of the same class and increases efficiency by limiting testing of multiple closely related agents. Equivalent agents are identified by:
  - Listing equivalent agents with an "or" in Tables 1 and 2. "Or" indicates cross-susceptibility and cross-resistance is nearly complete (very major error + major error < 3%; minor error < 10%) and only one agent needs to be tested.
  - Listing agents that are equivalent and results that can be deduced by testing the equivalent agent in a comment (see Tables 1 and 2).

The following tables include tests that fall into the supplemental, screening, surrogate agent, and equivalent agent test categories. The tables for supplemental, screening, and surrogate agent tests are comprehensive. The table for equivalent agent tests includes several examples, and many other equivalent agent tests are described throughout Tables 1 and 2.

Supplemental Tests (Required)

Supplemental Test	Organisms	Test Description	Required for:	Table Location
ICR	• *Staphylococcus* spp. • *Streptococcus pneumoniae* • *Streptococcus* spp. β-hemolytic group	Broth microdilution or disk diffusion with clindamycin and erythromycin tested together	Isolates that test erythromycin resistant and clindamycin susceptible or intermediate before reporting the isolate as clindamycin susceptible	3J
β-Lactamase	*Staphylococcus* spp.	Chromogenic cephalosporin (all staphylococci), penicillin disk diffusion zone-edge test (*S. aureus* only)	Isolates that test penicillin susceptible before reporting the isolate as penicillin susceptible	3G

Abbreviation: ICR, inducible clindamycin resistance.

Supplemental Tests (Optional)

Supplemental Test	Organisms	Test Description	Optional for:	Table Location
ESBL	• *Escherichia coli* • *K. pneumoniae* • *Klebsiella oxytoca* • *Proteus mirabilis*	Broth microdilution or disk diffusion clavulanate inhibition test for ESBLs	Isolates **meeting the criteria for testing as defined in Table 3A**	3A
Carba NP	• Enterobacterales • *P. aeruginosa*	Colorimetric assay for detecting carbapenem hydrolysis	Isolates that are not susceptible to one or more carbapenems	3B

Supplemental Tests (Optional) (Continued)

Supplemental Test	Organisms	Test Description	Optional for:	Table Location
mCIM with or without eCIM	• mCIM only: Enterobacterales and *P. aeruginosa* • mCIM with eCIM: Enterobacterales only	• Disk diffusion for detecting carbapenem hydrolysis (inactivation) • eCIM add-on enables differentiation of metallo-β-lactamases from serine carbapenemases in Enterobacterales isolates that are positive for mCIM	Isolates that are not susceptible to one or more carbapenems	3C
Aztreonam plus ceftazidime-avibactam broth disk elution	• Enterobacterales • *Stenotrophomonas maltophilia*	Tube dilution using aztreonam and ceftazidime-avibactam disks as antimicrobial agent source	Determining whether isolates are susceptible or not susceptible to the combination of aztreonam plus ceftazidime-avibactam	3D
Colistin agar test	• Enterobacterales • *P. aeruginosa*	Modified agar dilution	Determining the colistin MIC	3E
Colistin broth disk elution	• Enterobacterales • *P. aeruginosa*	Tube dilution using colistin disks as antimicrobial agent source	Determining the colistin MIC	3E
Oxacillin salt agar	*S. aureus*	Agar dilution; MHA with 4% NaCl and 6 µg/mL oxacillin	Detecting MRSA; see cefoxitin surrogate agent tests, which are preferred	3H

Abbreviations: Carba NP, carbapenemase Nordmann-Poirel; eCIM, EDTA-modified carbapenem inactivation method; EDTA, ethylenediaminetetraacetic acid; ESBL, extended-spectrum β-lactamase; mCIM, modified carbapenem inactivation method; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; MRSA, methicillin (oxacillin)-resistant *Staphylococcus aureus*; NaCl, sodium chloride.

Screening Tests

Screening Test	Organisms	Test Description	When to Perform Confirmatory Test	Confirmatory Test	Table Location
Vancomycin agar screen	• *S. aureus* • *Enterococcus* spp.	Agar dilution; BHI with 6 µg/mL vancomycin	If screen positive	Vancomycin MIC	3I
HLAR by disk diffusion	*Enterococcus* spp.	Disk diffusion with gentamicin and streptomycin	If screen inconclusive	Broth microdilution, agar dilution MIC	3L

Abbreviations: BHI, brain heart infusion; HLAR, high-level aminoglycoside resistance; MIC, minimal inhibitory concentration.

Surrogate Agent Tests

Surrogate Agent	Organisms	Test Description	Results	Table Locations
Cefazolin	• *E. coli* • *K. pneumoniae* • *P. mirabilis*	Broth microdilution or disk diffusion	When used for therapy of uncomplicated UTIs, predicts results for the following oral antimicrobial agents: cefaclor, cefdinir, cefpodoxime, cefprozil, cefuroxime, cephalexin, and loracarbef Cefazolin tested as a surrogate may overcall resistance to cefdinir, cefpodoxime, and cefuroxime. If cefazolin tests resistant, test these drugs individually if needed for therapy.	1A-1, 2A-1
Cefoxitin	• *S. aureus* • *Staphylococcus lugdunensis* • *Staphylococcus epidermidis* • Other *Staphylococcus* spp. (except *Staphylococcus pseudintermedius* and *Staphylococcus schleiferi*)	Broth microdilution: *S. aureus* *S. lugdunensis* Disk diffusion: *S. aureus* *S. lugdunensis* Other *Staphylococcus* spp., excluding *S. pseudintermedius* and *S. schleiferi*	Predicts results for *mecA*-mediated methicillin (oxacillin) resistance.	1C, 2C, 3H
Oxacillin	*S. pneumoniae*	Disk diffusion	Predicts penicillin susceptibility if oxacillin zone is ≥ 20 mm. If oxacillin zone is ≤ 19 mm, penicillin MIC must be performed.	1G, 2G
Pefloxacin	*Salmonella* spp.	Disk diffusion	Predicts reduced susceptibility to ciprofloxacin	2A-2

Abbreviations: MIC, minimal inhibitory concentration; UTI, urinary tract infection.

Examples of Equivalent Agent Tests

Agents	Organisms	Identified by	Table Locations
Cefotaxime or ceftriaxone	Enterobacterales, including *Salmonella* and *Shigella* spp.	"Or"	1A-1, 1A-2, 2A-1, and 2A-2
Colistin or polymyxin B	Enterobacterales, *P. aeruginosa*, *Acinetobacter baumannii* complex	"Or"	1B-2, 2A-1, 2B-1, and 2B-2
Azithromycin or clarithromycin or erythromycin	*Staphylococcus* spp.	"Or"	1C and 2C
Penicillin-susceptible staphylococci are susceptible to other β-lactam agents with established clinical efficacy for staphylococcal infections (including both penicillinase-labile and penicillinase-stable agents; see Glossary I). Penicillin-resistant staphylococci are resistant to penicillinase-labile penicillins.	*Staphylococcus* spp.	Note listed	1C and 2C
The results of ampicillin susceptibility tests should be used to predict the activity of amoxicillin.	*Haemophilus* spp.	Note listed	1E and 2E
The results of ampicillin susceptibility tests should be used to predict the activity of amoxicillin.	Anaerobes	Note listed	2J

IX. Quality Control and Verification

Recommendations for QC are included in various tables and appendixes. Acceptable ranges for QC strains are provided in Tables 4A-1 through 4B for disk diffusion and Tables 5A-1 through 5E for MIC testing. Guidance for QC frequency, **selection of QC strains**, and modifications of AST systems is found in Table 4C for disk diffusion and Table 5F for MIC testing. Guidance for troubleshooting out-of-range results is included in Table 4D for disk diffusion and Table 5G for MIC testing. Additional information is available in Appendix C (eg, QC organism characteristics, QC testing recommendations).

The Centers for Medicare & Medicaid Services (CMS) requires laboratories in the United States to perform appropriate QC testing for AST with each lot/batch or shipment of media and antimicrobial agent(s) before, or concurrent with initial use.[7] Thereafter, QC must be performed with each day of testing (subsequently referred to as "daily" QC testing). The specific QC strains required for daily QC testing are not specified by CMS. Other regulatory agencies may have alternative QC requirements. A laboratory in the US must develop an individualized quality control plan (IQCP) if it wishes to deviate from CMS's daily AST QC requirement. If an IQCP is acceptable to the laboratory's director and accreditation requirements, an IQCP can be designed to reduce AST QC frequency and to determine which QC strains to test. Refer to Appendix I for additional guidance on selection of QC strains and QC testing frequency.

Implementing any new diagnostic test requires verification.[8] Each laboratory that introduces a new AST system or adds a new antimicrobial agent to an existing AST system must verify or establish that, before reporting patient test results, the system meets performance specifications for that system. Verification generally involves testing patient isolates with the new AST system and comparing results to those obtained with an established reference method, a system that has been previously verified, **or in some cases the standard disk diffusion method**. Testing patient isolates may be done concurrently with the two systems. Alternatively, organisms with known MICs or zone sizes may be used for the verification. Guidance on verification studies is not included in CLSI M100. Other publications describe AST system verification (eg, CLSI M52[9] and Patel et al.[10]).

NOTE: Information in boldface type is new or modified since the previous edition.

X. Abbreviations and Acronyms

AR	antimicrobial resistance
AST	antimicrobial susceptibility testing
ATCC®[a]	American Type Culture Collection
BHI	brain heart infusion
BLNAR	β-lactamase negative, ampicillin-resistant
BMD	**broth microdilution**
BMHA	blood Mueller-Hinton agar
BSC	biological safety cabinet
BSL-2	biosafety level 2
BSL-3	biosafety level 3
CAMHB	cation-adjusted Mueller-Hinton broth
CAMHB-HSD	cation-adjusted Mueller-Hinton broth supplemented with horse serum (25% v/v) and 0.5 mM DL-dithiothreitol (pH 7.2–7.4)
Carba NP	carbapenemase Nordmann-Poirel
CAT	colistin agar test
CBDE	colistin broth disk elution
CFU	colony-forming unit(s)
CMS	**Centers for Medicare & Medicaid Services**
CO_2	carbon dioxide

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[a] ATCC® is a registered trademark of the American Type Culture Collection.

CSF	cerebrospinal fluid
d	day(s)
DMSO	dimethyl sulfoxide
DTT	DL-dithiothreitol
eCIM	EDTA-modified carbapenem inactivation method
ECV	epidemiological cutoff value
EDTA	ethylenediaminetetraacetic acid
ESBL	extended-spectrum β-lactamase
EUCAST	**European Committee on Antimicrobial Susceptibility Testing**
FDA	US Food and Drug Administration
GC	growth control
GC agar	gonococcus (*Neisseria gonorrhoeae*) agar
h	hour(s)
H_2O	water
HCl	hydrochloric acid
HLAR	high-level aminoglycoside resistance
HTM	*Haemophilus* test medium
I	intermediate
ICR	inducible clindamycin resistance
IM	intramuscular
Inv.	investigational agent
IQCP	**individualized quality control plan**
IV	intravenous
LHB	lysed horse blood
MALDI-TOF MS	matrix-assisted laser-desorption/ionization time-of-flight mass spectrometry
MBL	metallo-β-lactamase

mCIM	modified carbapenem inactivation method
MDRO	multidrug-resistant organism
MHA	Mueller-Hinton agar
MHB	Mueller-Hinton broth
MH-F	Mueller-Hinton fastidious
MIC	minimal inhibitory concentration
min	minute(s)
MRS	methicillin (oxacillin)-resistant staphylococci
MRSA	methicillin (oxacillin)-resistant *Staphylococcus aureus*
MSSA	methicillin (oxacillin)-susceptible *Staphylococcus aureus*
N/A	not applicable
NaCl	sodium chloride
NAD	β-nicotinamide adenine dinucleotide
NaOH	sodium hydroxide
NCTC	National Collection of Type Cultures
NS	nonsusceptible
PBP2a	penicillin-binding protein 2a
PCR	polymerase chain reaction
pH	negative logarithm of hydrogen ion concentration
PK/PD	pharmacokinetic/pharmacodynamic
PO	oral
q	quaque (every)
QC	quality control
R	resistant
S	susceptible
s	second(s)

SDD	susceptible-dose dependent
SOSA	**staphylococci other than *Staphylococcus aureus***
TSA	tryptic soy agar
TSB	trypticase soy broth
U	urine
UTI	urinary tract infection
VRE	vancomycin-resistant enterococci
wk	week(s)
y	year(s)
$ZnSO_4$	zinc sulfate

References

[1] CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests*. 14th ed. CLSI standard M02. Clinical and Laboratory Standards Institute; 2024.

[2] CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*. 12th ed. CLSI standard M07. Clinical and Laboratory Standards Institute; 2024.

[3] CLSI. *Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria*. 9th ed. CLSI standard M11. Clinical and Laboratory Standards Institute; 2018.

[4] CLSI. *Development of* In Vitro *Susceptibility Test Methods, Breakpoints, and Quality Control Parameters*. 6th ed. CLSI guideline M23. Clinical and Laboratory Standards Institute; 2023.

[5] CLSI. *Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria*. 3rd ed. CLSI guideline M45. Clinical and Laboratory Standards Institute; 2016.

[6] CLSI. *Analysis and Presentation of Cumulative Antimicrobial Susceptibility Test Data*. 5th ed. CLSI guideline M39. Clinical and Laboratory Standards Institute; 2022.

[7] **Centers for Medicare & Medicaid Services, US Department of Health and Human Services. Part 493—*Laboratory Requirements; Standard: Bacteriology* (Codified at 42 CFR §493.1261). Office of the Federal Register; published annually.**

[8] Centers for Medicare & Medicaid Services, US Department of Health and Human Services. *Part 493—Laboratory Requirements; Standard: Establishment and verification of performance specifications* (Codified at 42 CFR §493.1253). Office of the Federal Register; published annually.

[9] CLSI. *Verification of Commercial Microbial Identification and Antimicrobial Susceptibility Testing Systems*. 1st ed. CLSI guideline M52. Clinical and Laboratory Standards Institute; 2015.

[10] Patel JB, Sharp S, Novak-Weekley S. Verification of antimicrobial susceptibility testing methods: a practical approach. *Clin Microbiol Newslett*. 2013;35(13):103-109.doi:10.1016/j.clinmicnews.2013.06.001

Introduction to Tables 1A–1J. Antimicrobial Agents That Should Be Considered for Testing and Reporting by Microbiology Laboratories

Selecting the most appropriate antimicrobial agents to test and report is a decision best made by each laboratory in consultation with the antimicrobial stewardship team and other relevant institutional stakeholders. The suggestions in these tables:

- Include agents approved by the US Food and Drug Administration for clinical use
- Are directed toward medical laboratories in the United States but may be appropriate in other settings
- Are based on the understanding that patient-specific factors (eg, age, body site) or organism-specific factors (eg, overall antimicrobial susceptibility profile) must be considered for testing and reporting of any individual agent
- Need to be considered with institutional guidelines when used to develop a laboratory's testing and reporting protocols

Review the Instructions for Use of Tables and section I, Selecting Antimicrobial Agents for Testing and Reporting, for additional guidance regarding antimicrobial agent testing and reporting decisions, including the use of cascade and selective reporting strategies.

Refer to Tables 2 for zone diameter and minimal inhibitory concentration breakpoints, testing and reporting comments, and prediction comments such as when testing an antimicrobial agent can predict susceptibility to other agents (eg, tetracyclines).

"Warning": Do not report the following antimicrobial agents for bacteria isolated from CSF. These are not the drugs of choice and may not be effective for treating CSF infections caused by the bacteria included in Tables 2A through 2J:

- Agents administered by oral route only
- First- and second-generation cephalosporins and cephamycins
- Doripenem, ertapenem, and imipenem
- Clindamycin
- Lefamulin
- Macrolides
- Tetracyclines

Refer to Glossary I for individual agents within the drug classes listed above.

NOTE: Information in boldface type is new or modified since the previous edition.

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Table 1A-1. Enterobacterales (excluding *Salmonella* and *Shigella* spp.)[a]

Tier 1: Antimicrobial agents that are appropriate for routine, primary testing and reporting	**Tier 2: Antimicrobial agents that are appropriate for routine, primary testing but may be reported following cascade reporting rules established at each institution**	**Tier 3: Antimicrobial agents that are appropriate for routine, primary testing in institutions that serve patients at high risk for MDROs but should only be reported following cascade reporting rules established at each institution**	**Tier 4: Antimicrobial agents that may warrant testing and reporting by clinician request if antimicrobial agents in other tiers are not optimal because of various factors**
Ampicillin			
Cefazolin	Cefuroxime		
Cefotaxime or ceftriaxone[b]	Cefepime[c]		
	Ertapenem Imipenem Meropenem	Cefiderocol	
		Ceftazidime-avibactam	
		Imipenem-relebactam	
		Meropenem-vaborbactam	
Amoxicillin-clavulanate Ampicillin-sulbactam			
Piperacillin-tazobactam			
Gentamicin	Tobramycin	Plazomicin	
	Amikacin		
Ciprofloxacin Levofloxacin			
Trimethoprim-sulfamethoxazole			
	Cefotetan Cefoxitin		
	Tetracycline		
			Aztreonam[d]
			Ceftaroline[b]
			Ceftazidime[b]
			Ceftolozane-tazobactam
Urine Only			
Cefazolin (surrogate for uncomplicated UTI)[e]			
Nitrofurantoin			
		Fosfomycin[f] (*Escherichia coli*)	

Abbreviations: MDRO, multidrug-resistant organism; UTI, urinary tract infection.

Table 1A-1. Enterobacterales (Continued)

Footnotes

a. See Appendix B for species-specific intrinsic resistance profiles. If an antimicrobial agent–organism combination that is defined as intrinsically resistant is tested, the result should be reported as resistant. Consideration may be given to adding comments regarding intrinsic resistance of agents not tested.

b. *Citrobacter freundii* complex, *Enterobacter cloacae* complex, *Hafnia alvei, Klebsiella* (formerly *Enterobacter*) *aerogenes, Morganella morganii, Providencia* spp., *Serratia marcescens*, and *Yersinia enterocolitica* may test susceptible to ceftriaxone, cefotaxime, ceftazidime, and ceftaroline, but these agents may be ineffective against these genera within a few days after initiation of therapy due to derepression of inducible AmpC β-lactamase. The risk of AmpC derepression during therapy is moderate to high with *C. freundii* complex, *E. cloacae* complex, and *K. aerogenes* and appears to be less frequent with *M. morganii, Providencia* spp., and *S. marcescens*.[1] Therefore, isolates that are initially susceptible may become resistant. Testing subsequent isolates may be warranted if clinically indicated.

c. Cefepime should be considered a Tier 1 agent for testing and/or reporting of *C. freundii* complex, *E. cloacae* complex, *H. alvei, K. aerogenes, M. morganii*, *Providencia* spp., *S. marcescens*, and *Y. enterocolitica* (see footnote b).[1]

d. In institutions that serve patients at high risk for metallo-β-lactamase–producing Enterobacterales, aztreonam may be considered a Tier 3 agent following cascade reporting rules established at the institution.

e. See cefazolin comments in Table 2A-1 for using cefazolin as a surrogate test for oral cephalosporins and for reporting cefazolin when used for therapy in uncomplicated UTIs.

f. Report only on *E. coli* isolated from the urinary tract.

Reference for Table 1A-1

[1] Tamma PD, Aitken SL, Bonomo RA, Mathers AJ, van Duin D, Clancy CJ. IDSA 2024 guidance on the treatment of antimicrobial resistant gram-negative infections. Accessed 23 January 2024. https://www.idsociety.org/practice-guideline/amr-guidance/

NOTE: Information in boldface type is new or modified since the previous edition.

Table 1A-2. *Salmonella* and *Shigella* spp.[a,b]

Tier 1: Antimicrobial agents that are appropriate for routine, primary testing and reporting	Tier 2: Antimicrobial agents that are appropriate for routine, primary testing but may be reported following cascade reporting rules established at each institution	Tier 3: Antimicrobial agents that are appropriate for routine, primary testing in institutions that serve patients at high risk for MDROs but should only be reported following cascade reporting rules established at each institution	Tier 4: Antimicrobial agents that may warrant testing and reporting by clinician request if antimicrobial agents in other tiers are not optimal because of various factors
Ampicillin			
Ciprofloxacin Levofloxacin			
Trimethoprim-sulfamethoxazole			
Cefotaxime or ceftriaxone			Ertapenem[c] Imipenem[c] Meropenem[c]
	Azithromycin[d]		
			Tetracycline

Abbreviations: AST, antimicrobial susceptibility testing; MDRO, multidrug-resistant organism.

Footnotes

a. Table 2A-2 should be used for interpreting AST results for *Salmonella* and *Shigella* spp.

b. **WARNING:** For *Salmonella* and *Shigella* spp., aminoglycosides, first- and second-generation cephalosporins, and cephamycins may appear active *in vitro* but are not effective clinically and should not be reported as susceptible. Routine susceptibility testing is not indicated for nontyphoidal *Salmonella* spp. isolated from intestinal sources. However, susceptibility testing is indicated for all *Shigella* isolates. When fecal isolates of *Salmonella* and *Shigella* spp. are tested, only ampicillin, a fluoroquinolone, and trimethoprim-sulfamethoxazole should be reported routinely. In addition, for extraintestinal isolates of *Salmonella* spp., a third-generation cephalosporin should be tested and reported. Azithromycin may be tested and reported per institutional guidelines.

c. Ertapenem, imipenem, and/or meropenem might be considered for testing and/or reporting for isolates resistant to all agents in Tiers 1 and 2, although there are limited clinical data suggesting their effectiveness for treating salmonellosis or shigellosis.[1]

d. Report only on *Salmonella enterica* ser. Typhi and *Shigella* spp.

Reference for Table 1A-2

[1] CDC Health Alert Network. Extensively drug-resistant *Salmonella* Typhi infections among U.S. residents without international travel. Accessed 15 October 2024. https://emergency.cdc.gov/han/pdf/CDC-HAN-439-XDR-Salmonella-Typhi-Infections-in-U.S.-Without-Intl-Travel-02.12.2021.pdf

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Table 1B-1. *Pseudomonas aeruginosa*

Tier 1: Antimicrobial agents that are appropriate for routine, primary testing and reporting	Tier 2: Antimicrobial agents that are appropriate for routine, primary testing but may be reported following cascade reporting rules established at each institution	Tier 3: Antimicrobial agents that are appropriate for routine, primary testing in institutions that serve patients at high risk for MDROs but should only be reported following cascade reporting rules established at each institution	Tier 4: Antimicrobial agents that may warrant testing and reporting by clinician request if antimicrobial agents in other tiers are not optimal because of various factors
Ceftazidime	Imipenem Meropenem	Cefiderocol	
Cefepime		Ceftazidime-avibactam	
Piperacillin-tazobactam		Ceftolozane-tazobactam	
		Imipenem-relebactam	
Tobramycin			
Ciprofloxacin Levofloxacin			
			Aztreonam
Urine Only			
	Amikacin		

Abbreviation: MDRO, multidrug-resistant organism.

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Table 1B-2. *Acinetobacter* spp.

Tier 1: Antimicrobial agents that are appropriate for routine, primary testing and reporting	Tier 2: Antimicrobial agents that are appropriate for routine, primary testing but may be reported following cascade reporting rules established at each institution	Tier 3: Antimicrobial agents that are appropriate for routine, primary testing in institutions that serve patients at high risk for MDROs but should only be reported following cascade reporting rules established at each institution	Tier 4: Antimicrobial agents that may warrant testing and reporting by clinician request if antimicrobial agents in other tiers are not optimal because of various factors
Ampicillin-sulbactam			
Ceftazidime	Imipenem Meropenem	Cefiderocol	
Cefepime			
Ciprofloxacin Levofloxacin			
Gentamicin Tobramycin	Amikacin		
	Piperacillin-tazobactam		
	Trimethoprim-sulfamethoxazole		
	Minocycline		
		Sulbactam-durlobactam	
			Cefotaxime Ceftriaxone
			Colistin or polymyxin B

Abbreviation: MDRO, multidrug-resistant organism.

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Table 1B-3. *Burkholderia cepacia* Complex

Refer to Table 2B-3 and Appendix F for information regarding testing of *B. cepacia* complex.

NOTE: Information in boldface type is new or modified since the previous edition.

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Table 1B-4. *Stenotrophomonas maltophilia*

Tier 1: Antimicrobial agents that are appropriate for routine, primary testing and reporting	Tier 2: Antimicrobial agents that are appropriate for routine, primary testing but may be reported following cascade reporting rules established at each institution	Tier 3: Antimicrobial agents that are appropriate for routine, primary testing in institutions that serve patients at high risk for MDROs but should only be reported following cascade reporting rules established at each institution	Tier 4: Antimicrobial agents that may warrant testing and reporting by clinician request if antimicrobial agents in other tiers are not optimal because of various factors
Levofloxacin			
Minocycline			
Trimethoprim-sulfamethoxazole			
		Cefiderocol	

Abbreviation: MDRO, multidrug-resistant organism.

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Table 1B-5. Other Non-Enterobacterales[a,b]

Tier 1: Antimicrobial agents that are appropriate for routine, primary testing and reporting	Tier 2: Antimicrobial agents that are appropriate for routine, primary testing but may be reported following cascade reporting rules established at each institution	Tier 3: Antimicrobial agents that are appropriate for routine, primary testing in institutions that serve patients at high risk for MDROs but should only be reported following cascade reporting rules established at each institution	Tier 4: Antimicrobial agents that may warrant testing and reporting by clinician request if antimicrobial agents in other tiers are not optimal because of various factors
Ceftazidime	Cefepime		
	Imipenem Meropenem		
Gentamicin Tobramycin	Amikacin		
Piperacillin-tazobactam			
Trimethoprim-sulfamethoxazole			
	Aztreonam		
	Ciprofloxacin Levofloxacin		
	Minocycline		
			Cefotaxime Ceftriaxone
Urine Only			
Tetracycline			

Abbreviations: MDRO, multidrug-resistant organism; MIC, minimal inhibitory concentration.

Footnotes

a. Other non-Enterobacterales include *Pseudomonas* spp. and other nonfastidious, glucose-nonfermenting, gram-negative bacilli but exclude *Pseudomonas aeruginosa, Acinetobacter* spp., *Burkholderia cepacia* complex, and *Stenotrophomonas maltophilia*. Refer to each respective Table 1 for suggested antimicrobial agents to test and report.

b. MIC testing only; disk diffusion test is unreliable.

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Table 1C. *Staphylococcus* spp.

Tier 1: Antimicrobial agents that are appropriate for routine, primary testing and reporting	Tier 2: Antimicrobial agents that are appropriate for routine, primary testing but may be reported following cascade reporting rules established at each institution	Tier 3: Antimicrobial agents that are appropriate for routine, primary testing in institutions that serve patients at high risk for MDROs but should only be reported following cascade reporting rules established at each institution	Tier 4: Antimicrobial agents that may warrant testing and reporting by clinician request if antimicrobial agents in other tiers are not optimal because of various factors
Azithromycin or clarithromycin or erythromycin[a]			
Clindamycin[a]			
Oxacillin[b,c,d,e,f] Cefoxitin[b,c,d,e] (surrogate for oxacillin)		Ceftaroline[g]	
Doxycycline Minocycline[a] Tetracycline			
Trimethoprim-sulfamethoxazole			
Vancomycin[h]			
	Penicillin[b,i]		
	Daptomycin[h,j]		
	Linezolid	Tedizolid[g]	
		Rifampin[k]	
		Lefamulin[a,g]	
			Ciprofloxacin or levofloxacin Moxifloxacin
			Dalbavancin[g,h]
			Oritavancin[g,h]
			Telavancin[g,h]
			Gentamicin[l]
Urine Only			
Nitrofurantoin			

Abbreviations: MDRO, multidrug-resistant organism; MIC, minimal inhibitory concentration; MRS, methicillin (oxacillin)-resistant staphylococci.

Table 1C. *Staphylococcus* spp. (Continued)

Footnotes

a. Not routinely reported on organisms isolated from the urinary tract.

b. Penicillin-susceptible staphylococci are susceptible to other β-lactam agents with established clinical efficacy for staphylococcal infections (including both penicillinase-labile and penicillinase-stable agents; see Glossary I). Penicillin-resistant staphylococci are resistant to penicillinase-labile penicillins.

c. MRS are resistant to currently available β-lactam antimicrobial agents, with the exception of ceftaroline. Thus, susceptibility or resistance to a wide array of β-lactam antimicrobial agents may be deduced from testing only penicillin and either cefoxitin or oxacillin. Testing of other β-lactam agents, except ceftaroline, is not advised.

d. If a penicillinase-stable penicillin is tested, oxacillin is the preferred agent, and results can be applied to the other penicillinase-stable penicillins (refer to Glossary I). Detection of methicillin (oxacillin) resistance in staphylococci is achieved by using specific methods, as described in Tables 2C and 3H.

e. See oxacillin and cefoxitin comments in Table 2C for using cefoxitin as a surrogate test for oxacillin.

f. For *S. aureus, S. lugdunensis,* and other *Staphylococcus* spp. (except ***S. coagulans***, *S. epidermidis, S. pseudintermedius,* and *S. schleiferi*), only MIC testing for oxacillin, not disk diffusion testing, is acceptable; see exceptions in Table 2C.

g. For *S. aureus* only, including methicillin (oxacillin)-resistant *S. aureus*.

h. MIC testing only; disk diffusion testing is unreliable.

i. If penicillin is tested, confirm susceptible results before reporting (see Table 2C comment [9] and Table 3G).

j. Not routinely reported on organisms isolated from the lower respiratory tract.

k. ***Rx:*** Rifampin should not be used alone for antimicrobial therapy.

l. For staphylococci that test susceptible, gentamicin is used only in combination with other active agents that test susceptible.

NOTE: Information in boldface type is new or modified since the previous edition.

Table 1D. *Enterococcus* spp.[a]

Tier 1: Antimicrobial agents that are appropriate for routine, primary testing and reporting	Tier 2: Antimicrobial agents that are appropriate for routine, primary testing but may be reported following cascade reporting rules established at each institution	Tier 3: Antimicrobial agents that are appropriate for routine, primary testing in institutions that serve patients at high risk for MDROs but should only be reported following cascade reporting rules established at each institution	Tier 4: Antimicrobial agents that may warrant testing and reporting by clinician request if antimicrobial agents in other tiers are not optimal because of various factors
Ampicillin[b,c] Penicillin[c,d]			
	Vancomycin		
	Gentamicin[e] (high-level resistance testing only)	Streptomycin[e] (high-level resistance testing only)	
	Daptomycin[f,g]		
	Linezolid	Tedizolid	
			Dalbavancin[f,h]
			Oritavancin[f,h]
			Telavancin[f,h]
Urine Only			
Nitrofurantoin			
	Ciprofloxacin Levofloxacin		
		Fosfomycin[i]	
		Tetracycline	

Abbreviations: HLAR, high-level aminoglycoside resistance; MDRO, multidrug-resistant organism; MIC, minimal inhibitory concentration.

Table 1D. *Enterococcus* spp. (Continued)

Footnotes

a. **WARNING:** For *Enterococcus* spp., aminoglycosides (except for high-level resistance testing), cephalosporins, clindamycin, and trimethoprim-sulfamethoxazole may appear active *in vitro*, but are not effective clinically and should not be reported as susceptible.

b. The results of ampicillin susceptibility tests should be used to predict the activity of amoxicillin. Ampicillin results may be used to predict susceptibility to amoxicillin-clavulanate, ampicillin-sulbactam, and piperacillin-tazobactam among non–β-lactamase-producing enterococci. Ampicillin susceptibility can be used to predict imipenem susceptibility, provided the species is confirmed to be *E. faecalis*.

c. ***Rx:*** Combination therapy with high-dosage parenteral ampicillin, amoxicillin, penicillin, or vancomycin, plus an aminoglycoside, may be indicated for serious enterococcal infections such as endocarditis, unless high-level resistance to both gentamicin and streptomycin is documented; such combinations are predicted to result in synergistic killing of enterococci. Refer to Table 3L for HLAR testing.

d. Enterococci susceptible to penicillin are predictably susceptible to ampicillin, amoxicillin, ampicillin-sulbactam, amoxicillin-clavulanate, and piperacillin-tazobactam for non–β-lactamase-producing enterococci. However, enterococci susceptible to ampicillin cannot be assumed to be susceptible to penicillin. If penicillin results are needed, testing of penicillin is required.

e. See additional testing and reporting information in Table 3L.

f. MIC testing only; disk diffusion test is unreliable.

g. Not routinely reported on organisms isolated from the lower respiratory tract.

h. Report only on vancomycin-susceptible *E. faecalis*.

i. Report only on *E. faecalis* urinary tract isolates.

Table 1E. *Haemophilus influenzae* and *Haemophilus parainfluenzae*

Tier 1: Antimicrobial agents that are appropriate for routine, primary testing and reporting	**Tier 2: Antimicrobial agents that are appropriate for routine, primary testing but may be reported following cascade reporting rules established at each institution**	**Tier 3: Antimicrobial agents that are appropriate for routine, primary testing in institutions that serve patients at high risk for MDROs but should only be reported following cascade reporting rules established at each institution**	**Tier 4: Antimicrobial agents that may warrant testing and reporting by clinician request if antimicrobial agents in other tiers are not optimal because of various factors**
Ampicillin[a,b]	Cefotaxime or ceftazidime or ceftriaxone[a]	Meropenem[a]	Ertapenem or imipenem
	Ampicillin-sulbactam Amoxicillin-clavulanate[c]		
	Ciprofloxacin or levofloxacin or moxifloxacin		
	Trimethoprim-sulfamethoxazole		
			Azithromycin[c] Clarithromycin[c]
			Aztreonam
			Cefaclor[c] Cefprozil[c]
			Cefdinir or cefixime or cefpodoxime[c]
			Ceftolozane-tazobactam[d]
			Ceftaroline[d]
			Cefuroxime[c]
			Lefamulin[d]
			Rifampin[e]
			Tetracycline

Abbreviations: CSF, cerebrospinal fluid; MDRO, multidrug-resistant organism.

Table 1E. *Haemophilus influenzae* and *Haemophilus parainfluenzae* (Continued)

Footnotes

a. For isolates of *H. influenzae* from CSF, only results of testing with ampicillin, any of the third-generation cephalosporins listed, and meropenem are appropriate to report.

b. The results of ampicillin susceptibility tests should be used to predict the activity of amoxicillin. The majority of *H. influenzae* isolates that are resistant to ampicillin and amoxicillin produce a TEM-type β-lactamase. In most cases, a β-lactamase test can provide a rapid means of detecting resistance to ampicillin and amoxicillin.

c. Amoxicillin-clavulanate, azithromycin, cefaclor, cefdinir, cefixime, cefpodoxime, cefprozil, cefuroxime, and clarithromycin are used as empiric therapy for respiratory tract infections due to *Haemophilus* spp. The results of susceptibility tests with these antimicrobial agents are often not necessary for managing individual patients.

d. Report only on *H. influenzae*.

e. May be appropriate only for prophylaxis of case contacts. Refer to Table 2E.

Table 1F. *Neisseria gonorrhoeae*[a]

Tier 1: Antimicrobial agents that are appropriate for routine, primary testing and reporting	Tier 2: Antimicrobial agents that are appropriate for routine, primary testing but may be reported following cascade reporting rules established at each institution	Tier 3: Antimicrobial agents that are appropriate for routine, primary testing in institutions that serve patients at high risk for MDROs but should only be reported following cascade reporting rules established at each institution	Tier 4: Antimicrobial agents that may warrant testing and reporting by clinician request if antimicrobial agents in other tiers are not optimal because of various factors
Azithromycin			
Ceftriaxone Cefixime			
Ciprofloxacin			
Tetracycline			

Abbreviation: MDRO, multidrug-resistant organism.

Footnote

a. Culture and susceptibility testing of *N. gonorrhoeae* should be considered in cases of treatment failure. Antimicrobial agents recommended for testing include, at a minimum, the agents listed in Tier 1. The most current guidelines for treatment and testing are available from the Centers for Disease Control and Prevention.[1]

Reference for Table 1F

[1] Centers for Disease Control and Prevention. Gonorrhea: about gonorrhea. Accessed 15 October 2024. https://www.cdc.gov/gonorrhea/about/

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Table 1G. *Streptococcus pneumoniae*

Tier 1: Antimicrobial agents that are appropriate for routine, primary testing and reporting	Tier 2: Antimicrobial agents that are appropriate for routine, primary testing but may be reported following cascade reporting rules established at each institution	Tier 3: Antimicrobial agents that are appropriate for routine, primary testing in institutions that serve patients at high risk for MDROs but should only be reported following cascade reporting rules established at each institution	Tier 4: Antimicrobial agents that may warrant testing and reporting by clinician request if antimicrobial agents in other tiers are not optimal because of various factors
Erythromycin[a,b]			
Penicillin[c]			Amoxicillin[d] Amoxicillin-clavulanate[d]
Trimethoprim-sulfamethoxazole			
Cefotaxime[c,d] Ceftriaxone[c,d]			Cefepime[d]
			Ceftaroline
	Meropenem[c,d]		Ertapenem[d] Imipenem[d]
	Clindamycin[b]		
	Doxycycline Tetracycline		
	Levofloxacin[e] Moxifloxacin[e]		
	Vancomycin[c]		
			Lefamulin[b]
			Linezolid
			Cefuroxime[d]
			Rifampin[f]

Abbreviations: CSF, cerebrospinal fluid; MDRO, multidrug-resistant organism; MIC, minimal inhibitory concentration.

Table 1G. *Streptococcus pneumoniae* (Continued)

Footnotes

a. Susceptibility and resistance to azithromycin and clarithromycin can be predicted by testing erythromycin.

b. Not routinely reported on organisms isolated from the urinary tract.

c. Penicillin and cefotaxime, ceftriaxone, or meropenem should be tested by a reliable MIC method (such as that described in CLSI M07[1]) and reported routinely with *S. pneumoniae* isolated from CSF. Such isolates can also be tested against vancomycin using the MIC or disk diffusion method. With isolates from other sites, the oxacillin disk test may be used. If the oxacillin zone size is ≤ 19 mm, cefotaxime, ceftriaxone, meropenem, or penicillin MICs should be determined.

d. MIC testing only; disk diffusion test is unreliable.

e. Organisms that are susceptible to levofloxacin are also considered susceptible to gemifloxacin and moxifloxacin. However, some organisms that are intermediate or resistant to levofloxacin may be susceptible to gemifloxacin, moxifloxacin, or both.

f. ***Rx:*** Rifampin should not be used alone for antimicrobial therapy.

Reference for Table 1G

[1] CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically.* 12th ed. CLSI standard M07. Clinical and Laboratory Standards Institute; 2024.

Table 1H-1. *Streptococcus* spp. β-Hemolytic Group

Tier 1: Antimicrobial agents that are appropriate for routine, primary testing and reporting	**Tier 2: Antimicrobial agents that are appropriate for routine, primary testing but may be reported following cascade reporting rules established at each institution**	**Tier 3: Antimicrobial agents that are appropriate for routine, primary testing in institutions that serve patients at high risk for MDROs but should only be reported following cascade reporting rules established at each institution**	**Tier 4: Antimicrobial agents that may warrant testing and reporting by clinician request if antimicrobial agents in other tiers are not optimal because of various factors**
Clindamycin[a,b]			
Erythromycin[a,b,c]			
Penicillin[d] or ampicillin[d]		Cefotaxime or ceftriaxone	Cefepime
			Ceftaroline
	Tetracycline		
		Vancomycin	
			Linezolid Tedizolid[e]
			Daptomycin[f,g]
			Levofloxacin
			Dalbavancin[g,h]
			Oritavancin[g]
			Telavancin[g]

Abbreviations: FDA, US Food and Drug Administration; ICR, inducible clindamycin resistance; MDRO, multidrug-resistant organism; MIC, minimal inhibitory concentration.

Table 1H-1. *Streptococcus* spp. β-Hemolytic Group (Continued)

Footnotes

a. Not routinely reported for organisms isolated from urinary tract.

b. ***Rx:*** Recommendations for intrapartum prophylaxis for group B streptococci are penicillin or ampicillin. Although cefazolin is recommended for penicillin-allergic women at low risk for anaphylaxis, those at high risk for anaphylaxis may receive clindamycin or vancomycin (if the isolate is not susceptible to clindamycin).[1] Group B streptococci are susceptible to ampicillin, penicillin, and cefazolin but may be resistant to erythromycin and clindamycin. When clindamycin is being considered for intrapartum prophylaxis (eg, pregnant woman with severe penicillin allergy), erythromycin and clindamycin (including ICR) should be tested, but only clindamycin should be reported. See Table 3J.

c. Susceptibility and resistance to azithromycin and clarithromycin can be predicted by testing erythromycin.

d. Penicillin and ampicillin are drugs of choice for treating β-hemolytic streptococcal infections. Susceptibility testing of penicillins and other β-lactams approved by the FDA for treatment of β-hemolytic streptococcal infections does not need to be performed routinely, because nonsusceptible isolates (ie, penicillin MICs > 0.12 and ampicillin MICs > 0.25 µg/mL) are extremely rare in any β-hemolytic streptococci and have not been reported for *S. pyogenes*. If testing is performed, any β-hemolytic streptococcal isolate found to be nonsusceptible should be re-identified, retested, and if confirmed, submitted to a public health laboratory (see Appendix A for additional instructions).

e. Report only on *S. pyogenes* and *S. agalactiae*.

f. Not routinely reported on organisms isolated from the lower respiratory tract.

g. MIC testing only; disk diffusion test is unreliable.

h. Report only on *S. pyogenes, S. agalactiae,* and *S. dysgalactiae*.

Reference for Table 1H-1

[1] American College of Obstetricians and Gynecologists. Prevention of group B streptococcal early-onset disease in newborns: ACOG Committee Opinion, Number 797. *Obstet Gynecol.* 2020;135(2):e51-e72. doi:10.1097/AOG.0000000000003668

Table 1H-2. *Streptococcus* spp. Viridans Group

Tier 1: Antimicrobial agents that are appropriate for routine, primary testing and reporting	Tier 2: Antimicrobial agents that are appropriate for routine, primary testing but may be reported following cascade reporting rules established at each institution	Tier 3: Antimicrobial agents that are appropriate for routine, primary testing in institutions that serve patients at high risk for MDROs but should only be reported following cascade reporting rules established at each institution	Tier 4: Antimicrobial agents that may warrant testing and reporting by clinician request if antimicrobial agents in other tiers are not optimal because of various factors
Ampicillin[a,b] Penicillin[a,b]			
Cefotaxime Ceftriaxone			Cefepime
	Vancomycin		
		Linezolid Tedizolid[c]	
		Dalbavancin[a,c]	
		Oritavancin[a]	
		Telavancin[a]	
			Ceftolozane-tazobactam
			Clindamycin[d]
			Erythromycin[d,e]
			Levofloxacin

Abbreviations: MDRO, multidrug-resistant organism; MIC, minimal inhibitory concentration.

Footnotes

a. MIC testing only; disk diffusion test is unreliable.

b. ***Rx:*** Penicillin- or ampicillin-intermediate isolates may necessitate combined therapy with an aminoglycoside for bactericidal action.

c. Report only on *S. anginosus* group (including *S. anginosus, S. intermedius*, and *S. constellatus*).

d. Not routinely reported on organisms isolated from urinary tract.

e. Susceptibility and resistance to azithromycin and clarithromycin can be predicted by testing erythromycin.

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Table 1I. *Neisseria meningitidis*[a,b]

Tier 1: Antimicrobial agents that are appropriate for routine, primary testing and reporting	Tier 2: Antimicrobial agents that are appropriate for routine, primary testing but may be reported following cascade reporting rules established at each institution	Tier 3: Antimicrobial agents that are appropriate for routine, primary testing in institutions that serve patients at high risk for MDROs but should only be reported following cascade reporting rules established at each institution	Tier 4: Antimicrobial agents that may warrant testing and reporting by clinician request if antimicrobial agents in other tiers are not optimal because of various factors
Penicillin			
Cefotaxime or ceftriaxone			Meropenem
			Azithromycin[c]
			Ciprofloxacin[c] Levofloxacin[c]
			Minocycline[c]
			Trimethoprim-sulfamethoxazole[d]
			Rifampin[c]

Abbreviations: AST, antimicrobial susceptibility testing; BSC, biological safety cabinet; BSL-2, biosafety level 2; BSL-3, biosafety level 3; MDRO, multidrug-resistant organism.

Table 1I. *Neisseria meningitidis* (Continued)

Footnotes

a. Important: For complete information on safety precautions, see *Biosafety in Microbiological and Biomedical Laboratories*. 6th ed. Centers for Disease Control and Prevention; 2020. Accessed 15 October 2024. https://www.cdc.gov/labs/pdf/SF__19_308133-A_BMBL6_00-BOOK-WEB-final-3.pdf

b. Recommended precautions: Perform all AST of *N. meningitidis* in a BSC. Manipulating *N. meningitidis* outside a BSC is associated with increased risk for contracting meningococcal disease. Laboratory-acquired meningococcal disease is associated with a case fatality rate of 50%. Exposure to droplets or aerosols of *N. meningitidis* is the most likely risk for laboratory-acquired infection. Rigorous protection from droplets or aerosols is mandated when microbiological procedures (including AST) are performed on all *N. meningitidis* isolates.

   If a BSC is unavailable, manipulation of these isolates should be minimized, limited to Gram staining or serogroup identification using phenolized saline solution, while wearing a laboratory coat and gloves and working behind a full-face splash shield. Use BSL-3 practices, procedures, and containment equipment for activities with a high potential for droplet or aerosol production and for activities involving production quantities or high concentrations of infectious materials. If BSL-2 or BSL-3 facilities are not available, forward isolates to a referral or public health laboratory with a minimum of BSL-2 facilities.

c. May be appropriate only for prophylaxis of meningococcal case contacts. These breakpoints do not apply to therapy of patients with invasive meningococcal disease.

d. Trimethoprim-sulfamethoxazole is the preferred disk for detection of sulfonamide resistance. Trimethoprim-sulfamethoxazole testing predicts susceptibility and resistance to trimethoprim-sulfamethoxazole and sulfonamides. Sulfonamides may be appropriate only for prophylaxis of meningococcal case contacts.

Table 1J. Anaerobes

Tier 1: Antimicrobial agents that are appropriate for routine, primary testing and reporting	Tier 2: Antimicrobial agents that are appropriate for routine, primary testing but may be reported following cascade reporting rules established at each institution	Tier 3: Antimicrobial agents that are appropriate for routine, primary testing in institutions that serve patients at high risk for MDROs but should only be reported following cascade reporting rules established at each institution	Tier 4: Antimicrobial agents that may warrant testing and reporting by clinician request if antimicrobial agents in other tiers are not optimal because of various factors
Ampicillin (gram-positive anaerobes)[a,b] Penicillin (gram-positive anaerobes)[a,b,c]			Ampicillin (gram-negative anaerobes)[a,b] Penicillin (gram-negative anaerobes)[a,b,c]
Amoxicillin-clavulanate Ampicillin-sulbactam Piperacillin-tazobactam			
Clindamycin			
Ertapenem Imipenem[d] Meropenem			Imipenem-relebactam[d]
Metronidazole[e]			
			Cefotetan Cefoxitin
			Ceftriaxone
			Moxifloxacin
			Tetracycline

Abbreviation: MDRO, multidrug-resistant organism.

Table 1J. Anaerobes (Continued)

Footnotes

a. Ampicillin and penicillin are Tier 1 agents for gram-positive anaerobes, most of which are β-lactamase negative. Ampicillin and penicillin are Tier 4 agents for gram-negative anaerobes, many of which are β-lactamase positive.

b. If either gram-positive or gram-negative isolates are β-lactamase positive, report as resistant to penicillin and ampicillin. Be aware that β-lactamase–negative isolates may be resistant to penicillin and ampicillin by other mechanisms.

c. Penicillin retains good *in vitro* activity against most Fusobacterium spp. and may be considered for primary testing and reporting with this genus. **β-Lactamases have been reported in this genus.**

d. Organisms that test susceptible to imipenem are also considered susceptible to imipenem-relebactam. However, organisms that test susceptible to imipenem-relebactam cannot be assumed to be susceptible to imipenem.

e. Many non–spore-forming, gram-positive anaerobic rods are resistant to metronidazole

NOTE 1: Most anaerobic infections are polymicrobial, including both β-lactamase–positive and β-lactamase–negative strains. Testing may not be necessary for isolates associated with polymicrobial anaerobic infections. However, if susceptibility testing is requested, only the organism most likely to be resistant (eg, *Bacteroides* and *Parabacteroides* spp.) should be tested and results reported (see Appendix D).

NOTE 2: Specific *Clostridium* spp. (eg, *Clostridium septicum, Paeniclostridium sordellii*) may be the singular cause of infection and are typically susceptible to penicillin and ampicillin. Penicillin and clindamycin resistance have been reported in *Clostridium perfringens*. Agents in Tier 1 of this table should be tested and reported for *Clostridium* spp.

NOTE 3: Information in boldface type is new or modified since the previous edition.

Introduction to Tables 2A–2J. Zone Diameter and MIC Breakpoints

Tables 2A through 2J: Tables for each organism group contain:

- Recommended testing conditions
- QC recommendations (see also CLSI M02[1] and CLSI M07[2])
- General comments for testing and/or reporting the organism group and specific comments for testing and/or reporting particular agent-organism combinations
- Zone diameter and minimal inhibitory concentration breakpoints

Tables 2 should be used with Tables 1 when deciding which agents to test and report. Specific symbols and notations are added to some antimicrobial agents to highlight additional considerations for reporting and/or explain their absence from Tables 1.

Table 2 Dosages. Antimicrobial Agent Dosage Regimens Used to Establish Susceptible or Susceptible-Dose Dependent Breakpoints:

- Recently approved susceptible or susceptible-dose dependent breakpoints for a number of agents are based on a specific dosage regimen(s); these dosage regimens are listed in this table, which follows Table 2J and precedes Tables 3.
- Dosage regimen information listed in this table should be shared with pharmacists, infectious diseases staff, and others making dosing recommendations for the institution.

It is important to review the Instructions for Use of Tables for additional guidance regarding antimicrobial agent testing and reporting, breakpoint and interpretive category definitions, and more information on use of content throughout Tables 2.

Introduction to Tables 2A–2J. (Continued)

"Warning": Do not report the following antimicrobial agents for bacteria isolated from CSF. These are not the drugs of choice and may not be effective for treating CSF infections caused by the bacteria included in Tables 2A through 2J:

- Agents administered by oral route only
- First- and second-generation cephalosporins and cephamycins
- Doripenem, ertapenem, and imipenem
- Clindamycin
- Lefamulin
- Macrolides
- Tetracyclines

Refer to Glossary I for individual agents within the drug classes listed above.

References for Introduction to Tables 2A–2J

[1] CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests*. 14th ed. CLSI standard M02. Clinical and Laboratory Standards Institute; 2024.

[2] CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*. 12th ed. CLSI standard M07. Clinical and Laboratory Standards Institute; 2024.

Table 2A-1. Zone Diameter and MIC Breakpoints for Enterobacterales (excluding *Salmonella* and *Shigella* spp.)

Testing Conditions

Medium: Disk diffusion: MHA
Broth dilution: CAMHB; iron-depleted CAMHB for cefiderocol (see Appendix H, **section H1**)[1]
Agar dilution: MHA

Inoculum: Broth culture method or colony suspension, equivalent to a 0.5 McFarland standard; positive blood culture broth for select antimicrobial agents with disk diffusion (see general comment [4])

Incubation: 35°C ± 2°C; ambient air
Disk diffusion: 16–18 hours
Dilution methods: 16–20 hours

QC Recommendations

Refer to the following:

- **Tables 4A-1, 4A-2, 5A-1, and 5A-2 that list acceptable QC ranges applicable for each method**
- **Appendix I to develop a QC plan**

When a commercial test system is used for antimicrobial susceptibility testing, refer to the manufacturer's instructions for QC **strains** and QC ranges.

Refer to Tables 3A, 3B, 3C, 3D, 3E, 3F-1, and 3F-2 for additional testing, reporting, and QC for Enterobacterales.

General Comments

(1) Refer to Table 1A-1 for antimicrobial agents that should be considered for testing and reporting by microbiology laboratories.

(2) For disk diffusion, test a maximum of 12 disks on a 150-mm plate and no more than 6 disks on a 100-mm plate; disks should be placed no less than 24 mm apart, center to center (see CLSI M02[2]). Each zone diameter should be clearly measurable; overlapping zones prevent accurate measurement. Measure the diameter of the zones of complete inhibition (as judged by the unaided eye), including the diameter of the disk (see CLSI M02QG[3]). Hold the Petri plate a few inches above a black background illuminated with reflected light. The zone margin should be considered the area showing no obvious, visible growth that can be detected with the unaided eye. Ignore faint growth of tiny colonies that can be detected only with a magnifying lens at the edge of the zone of inhibited growth. Strains of *Proteus* spp. may swarm into areas of inhibited growth around certain antimicrobial agents. With *Proteus* spp., ignore the thin veil of swarming growth in an otherwise obvious zone of growth inhibition. With trimethoprim and the sulfonamides, antagonists in the medium may allow some slight growth; therefore, disregard slight growth (20% or less of the lawn of growth) and measure the more obvious margin to determine the zone diameter.

(3) An intermediate (I) with a ^ in Tables 2 indicates agents that have the potential to concentrate in the urine. The I^ is for informational use only. The decision to report I^ is best made by each laboratory based on institution-specific guidelines and in consultation with appropriate medical personnel.

Table 2A-1. Enterobacterales (excluding *Salmonella* and *Shigella* spp.) (Continued)

(4) Positive blood culture broth can be used as the inoculum for direct disk diffusion testing of select antimicrobial agents against Enterobacterales (using methods described in Table 3F-1 and applying breakpoints in Table 3F-2). For antimicrobial agents not listed in Table 3F-2 for Enterobacterales, CLSI has not yet evaluated this direct disk diffusion method.

NOTE: Information in boldface type is new or modified since the previous edition.

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm				Interpretive Categories and MIC Breakpoints, µg/mL				Comments
		S	SDD	I	R	S	SDD	I	R	
PENICILLINS										
Ampicillin	10 µg	≥ 17	–	14–16^	≤ 13	≤ 8	–	16^	≥ 32	**(5)** Results of ampicillin testing can be used to predict results for amoxicillin. **(6)** Breakpoints when oral ampicillin is used are only for therapy of uncomplicated UTIs due to *Escherichia coli* and *Proteus mirabilis*.
Piperacillin*		–	–	–	–	≤ 8	16	–	≥ 32	**(7)** Disk diffusion breakpoints have been removed because no disk correlate data are available for the revised piperacillin MIC breakpoints. Disk diffusion breakpoints will be reassessed if data become available.
Mecillinam* (U)[a]	10 µg	≥ 15	–	12–14^	≤ 11	≤ 8	–	16^	≥ 32	**(8)** Report only on *E. coli*.
β-LACTAM COMBINATION AGENTS										
(9) Organisms that test susceptible to the β-lactam agent alone are also considered susceptible to the β-lactam combination agent. However, organisms that test susceptible to the β-lactam combination agent cannot be assumed to be susceptible to the β-lactam agent alone. Similarly, organisms that test SDD, intermediate, or resistant to the β-lactam agent alone may be susceptible to the β-lactam combination agent.										
Amoxicillin-clavulanate	20/10 µg	≥ 18	–	14–17^	≤ 13	≤ 8/4	–	16/8^	≥ 32/16	**(10)** Breakpoints when oral amoxicillin-clavulanate is used are only for therapy of uncomplicated UTIs or for completion of therapy for systemic infection.
Ampicillin-sulbactam	10/10 µg	≥ 15	–	12–14^	≤ 11	≤ 8/4	–	16/8^	≥ 32/16	
Ceftolozane-tazobactam	30/10 µg	≥ 22	–	19–21^	≤ 18	≤ 2/4	–	4/4^	≥ 8/4	

Table 2A-1. Enterobacterales (excluding *Salmonella* and *Shigella* spp.) (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm				Interpretive Categories and MIC Breakpoints, µg/mL				Comments
		S	SDD	I	R	S	SDD	I	R	
β-LACTAM COMBINATION AGENTS (Continued)										
Ceftazidime-avibactam	30/20 µg	≥ 21	–	–	≤ 20	≤ 8/4	–	–	≥ 16/4	**(11)** Confirmatory MIC testing is indicated for isolates with zones of 20–22 mm to avoid reporting false-susceptible or false-resistant results.
Imipenem-relebactam	10/25 µg	≥ 25	–	21–24^	≤ 20	≤ 1/4	–	2/4^	≥ 4/4	**(12)** Breakpoints do not apply to the family Morganellaceae, which includes but is not limited to the genera *Morganella*, *Proteus*, and *Providencia*.
Meropenem-vaborbactam	20/10 µg	≥ 18	–	15–17^	≤ 14	≤ 4/8	–	8/8^	≥ 16/8	**(13)** Enterobacterales that harbor OXA-48–like enzymes may test susceptible to meropenem-vaborbactam but may not respond to meropenem-vaborbactam *in vivo*. If an OXA-48–like gene or enzyme is detected, suppress meropenem-vaborbactam or report as resistant.
Piperacillin-tazobactam	100/10 µg	≥ 25	21–24	–	≤ 20	≤ 8/4	16/4	–	≥ 32/4	
Ticarcillin-clavulanate*	75/10 µg	≥ 20	–	15–19^	≤ 14	≤ 16/2	–	32/2–64/2^	≥ 128/2	

Table 2A-1. Enterobacterales (excluding *Salmonella* and *Shigella* spp.) (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm				Interpretive Categories and MIC Breakpoints, µg/mL				Comments
		S	SDD	I	R	S	SDD	I	R	
CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.)										
(14) Following evaluation of PK/PD properties, limited clinical data, and MIC distributions, revised breakpoints for cephalosporins (cefazolin, cefotaxime, ceftazidime, ceftizoxime, and ceftriaxone) and aztreonam were first published in January 2010 (CLSI M100-S20) and are listed in this table. Cefuroxime (parenteral) was also evaluated; however, no change in breakpoints was necessary for the dosage listed in Table 2 Dosages. When using current breakpoints, routine ESBL testing is not necessary before reporting results. However, in consultation with the antimicrobial stewardship team and other relevant institutional stakeholders, laboratories may decide to perform phenotypic or genotypic testing for ESBLs, and the results may be used to guide therapeutic management or for epidemiological or infection prevention purposes. Limitations of phenotypic and genotypic methods must be considered (see Table 3A introductory text).[4] Breakpoints for drugs with limited availability in many countries (eg, moxalactam, cefonicid, cefamandole, and cefoperazone) were not evaluated. If considering use of these drugs for *E. coli, Klebsiella pneumoniae* and *Klebsiella oxytoca*, or *Proteus* spp., ESBL testing should be performed (see Table 3A). If isolates test ESBL positive, the results for moxalactam, cefonicid, cefamandole, and cefoperazone should be reported as resistant.										
(15) Some Enterobacterales may develop resistance during therapy with third-generation cephalosporins as a result of derepression of AmpC β-lactamase. This derepression is most commonly seen with *Citrobacter freundii* complex, *Enterobacter cloacae* complex, and *Klebsiella* (formerly *Enterobacter*) *aerogenes*. Isolates that are initially susceptible may become resistant within a few days after initiation of therapy. Testing subsequent isolates may be warranted if clinically indicated. The approach to reporting AST results for these organisms should be determined in consultation with the antimicrobial stewardship team and other relevant institutional stakeholders. See Table 1A-1, footnotes b and c.[4]										
Cefazolin	30 µg	≥ 23	–	20–22	≤ 19	≤ 2	–	4	≥ 8	**(16)** Breakpoints when cefazolin is used for therapy of infections other than uncomplicated UTIs due to *E. coli, K. pneumoniae,* and *P. mirabilis*. See comment (14).
Cefazolin (U)[a]	30 µg	≥ 15	–	–	≤ 14	≤ 16	–	–	≥ 32	**(17)** Breakpoints when cefazolin is used for therapy of uncomplicated UTIs due to *E. coli, K. pneumoniae,* and *P. mirabilis*. See additional information in CEPHEMS (ORAL).
Ceftaroline	30 µg	≥ 23	–	20–22^	≤ 19	≤ 0.5	–	1^	≥ 2	

Table 2A-1. Enterobacterales (excluding *Salmonella* and *Shigella* spp.) (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm				Interpretive Categories and MIC Breakpoints, µg/mL				Comments
		S	SDD	I	R	S	SDD	I	R	
CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.) (Continued)										
Cefepime	30 µg	≥ 25	19–24	–	≤ 18	≤ 2	4–8	–	≥ 16	**(18)** Cefepime S/SDD results should be suppressed or edited and reported as resistant for isolates that demonstrate carbapenemase production (see Appendix G, Table G3).
Cefotaxime or	30 µg	≥ 26	–	23–25^	≤ 22	≤ 1	–	2^	≥ 4	See comment (14).
ceftriaxone	30 µg	≥ 23	–	20–22^	≤ 19	≤ 1	–	2^	≥ 4	
Cefotetan	30 µg	≥ 16	–	13–15^	≤ 12	≤ 16	–	32^	≥ 64	
Cefoxitin	30 µg	≥ 18	–	15–17^	≤ 14	≤ 8	–	16^	≥ 32	
Cefuroxime (parenteral)	30 µg	≥ 18	–	15–17^	≤ 14	≤ 8	–	16^	≥ 32	See comment (14).
Ceftazidime	30 µg	≥ 21	–	18–20^	≤ 17	≤ 4	–	8^	≥ 16	See comment (14).
Cefamandole*	30 µg	≥ 18	–	15–17^	≤ 14	≤ 8	–	16^	≥ 32	See comment (14).
Cefmetazole*	30 µg	≥ 16	–	13–15^	≤ 12	≤ 16	–	32^	≥ 64	**(19)** Insufficient new data exist to reevaluate breakpoints listed here.
Cefonicid*	30 µg	≥ 18	–	15–17^	≤ 14	≤ 8	–	16^	≥ 32	See comment (14).
Cefoperazone*	75 µg	≥ 21	–	16–20	≤ 15	≤ 16	–	32	≥ 64	See comment (14).
Ceftizoxime*	30 µg	≥ 25	–	22–24^	≤ 21	≤ 1	–	2^	≥ 4	See comment (14).
Moxalactam*	30 µg	≥ 23	–	15–22^	≤ 14	≤ 8	–	16–32^	≥ 64	See comment (14).
Cefiderocol	30 µg	≥ 16	–	9–15^	≤ 8	≤ 4	–	8^	≥ 16	**(20)** The accuracy and reproducibility of cefiderocol testing results by disk diffusion and broth microdilution are markedly affected by iron concentration and inoculum preparation and may vary by disk and media manufacturer. Depending on the type of variance observed, false-resistant or false-susceptible results may occur. Testing subsequent isolates is encouraged. Discussion with prescribers and antimicrobial stewardship members regarding the potential for inaccuracies is recommended.

Table 2A-1. Enterobacterales (excluding *Salmonella* and *Shigella* spp.) (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm				Interpretive Categories and MIC Breakpoints, µg/mL				Comments
		S	SDD	I	R	S	SDD	I	R	
CEPHEMS (ORAL)										
Cefazolin (U)[a] (surrogate test for oral cephalosporins and uncomplicated UTIs)	30 µg	≥ 15	–	–	≤ 14	≤ 16	–	–	≥ 32	**(21)** Breakpoints are for cefazolin when used as a surrogate test to predict results for the oral agents cefaclor, cefdinir, cefpodoxime, cefprozil, cefuroxime, cephalexin, and loracarbef when used for therapy of uncomplicated UTIs due to *E. coli*, *K. pneumoniae*, and *P. mirabilis*. Cefazolin tested as a surrogate may overcall resistance to cefdinir, cefpodoxime, and cefuroxime. If cefazolin tests resistant, test these drugs individually if needed for therapy.
Cefuroxime (oral)	30 µg	≥ 23	–	15–22^	≤ 14	≤ 4	–	8–16^	≥ 32	See comment (21).
Loracarbef*	30 µg	≥ 18	–	15–17^	≤ 14	≤ 8	–	16^	≥ 32	**(22)** Do not test *Citrobacter*, *Providencia*, or *Enterobacter* spp. with cefdinir or loracarbef by disk diffusion because false-susceptible results have been reported. See comment (21).
Cefaclor*	30 µg	≥ 18	–	15–17^	≤ 14	≤ 8	–	16^	≥ 32	See comment (21).
Cefdinir*	5 µg	≥ 20	–	17–19^	≤ 16	≤ 1	–	2^	≥ 4	See comments (21) and (22).
Cefixime*	5 µg	≥ 19	–	16–18^	≤ 15	≤ 1	–	2^	≥ 4	**(23)** Do not test *Morganella* spp. with cefixime, cefpodoxime, or cefetamet by disk diffusion.
Cefpodoxime*	10 µg	≥ 21	–	18–20^	≤ 17	≤ 2	–	4^	≥ 8	See comments (21) and (23).
Cefprozil*	30 µg	≥ 18	–	15–17^	≤ 14	≤ 8	–	16^	≥ 32	**(24)** Do not test *Providencia* spp. with cefprozil by disk diffusion because false-susceptible results have been reported. See comment (21).
Cefetamet (Inv.)	10 µg	≥ 18	–	15–17^	≤ 14	≤ 4	–	8^	≥ 16	See comment (23).
Ceftibuten (U, Inv.)[a]	30 µg	≥ 21	–	18–20^	≤ 17	≤ 8	–	16^	≥ 32	
MONOBACTAMS										
Aztreonam	30 µg	≥ 21	–	18–20^	≤ 17	≤ 4	–	8^	≥ 16	See comment (14).

Table 2A-1. Enterobacterales (excluding *Salmonella* and *Shigella* spp.) (Continued)

CARBAPENEMS

(25) Following evaluation of PK/PD properties, limited clinical data, and MIC distributions that include recently described carbapenemase-producing strains, revised breakpoints for carbapenems were first published in June 2010 (CLSI M100-S20-U) and are listed below. Because of limited treatment options for infections caused by organisms with carbapenem MICs or zone diameters in the intermediate range, clinicians may wish to design carbapenem dosage regimens that use maximum recommended doses and possibly prolonged IV infusion regimens, as has been reported in the literature.[5-8] Consultation with an infectious diseases specialist is recommended for isolates for which the carbapenem MICs or zone diameter results from disk diffusion testing are in the intermediate or resistant ranges.

Isolates resistant to any carbapenem tested (eg, ertapenem, imipenem, meropenem) should be tested for a carbapenemase using phenotypic and/or molecular assays. An exception to this recommendation is *Proteus, Providencia,* and *Morganella* spp. that are only resistant to imipenem. These assays should identify and ideally differentiate the presence of specific carbapenemase types (eg, KPC, NDM, OXA-48, VIM, IMP).

Decisions related to carbapenemase testing and reporting are best made by each laboratory in consultation with the antimicrobial stewardship team and other relevant institutional stakeholders.

These results do not replace antimicrobial susceptibility testing, but are important for treatment decisions, and to inform infection control and prevention interventions and/or epidemiologic investigations.

Depending on local epidemiology and available resources, carbapenemase testing for *E. cloacae* complex and *K. aerogenes* isolates that are only resistant to ertapenem might not be necessary. Ertapenem resistance in these species is often due to mechanisms other than carbapenemase production and carbapenemases are currently uncommon in such isolates.

See Appendix G, Table G3 regarding suggestions for reporting when mechanism of resistance-based testing (molecular and phenotypic methods) is discordant with phenotypic AST.

The following information is provided as background on carbapenemases in Enterobacterales that are largely responsible for MICs and zone diameters in the intermediate and resistant ranges, and thus the rationale for setting revised carbapenem breakpoints:

- The clinical effectiveness of carbapenem treatment of infections produced by isolates for which the carbapenem MIC or disk diffusion test results are within the intermediate range is uncertain due to lack of controlled clinical studies.

Imipenem MICs for *Proteus* spp., *Providencia* spp., and *Morganella morganii* tend to be higher (eg, MICs in the intermediate or resistant range) than meropenem or doripenem MICs. These isolates may have elevated imipenem MICs by mechanisms other than production of carbapenemases.

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm				Interpretive Categories and MIC Breakpoints, µg/mL				Comments
		S	SDD	I	R	S	SDD	I	R	
Doripenem*	10 µg	≥ 23	–	20–22^	≤ 19	≤ 1	–	2^	≥ 4	
Ertapenem	10 µg	≥ 22	–	19–21^	≤ 18	≤ 0.5	–	1^	≥ 2	
Imipenem	10 µg	≥ 23	–	20–22^	≤ 19	≤ 1	–	2^	≥ 4	
Meropenem	10 µg	≥ 23	–	20–22^	≤ 19	≤ 1	–	2^	≥ 4	

Table 2A-1. Enterobacterales (excluding *Salmonella* and *Shigella* spp.) (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm				Interpretive Categories and MIC Breakpoints, µg/mL				Comments
		S	SDD	I	R	S	SDD	I	R	
LIPOPEPTIDES										
(26) WARNING: Clinical and PK/PD data demonstrate colistin and polymyxin B have limited clinical efficacy, even if an intermediate result is obtained. Alternative agents are strongly preferred. Colistin and polymyxin B should be used in combination with one or more active antimicrobial agents. Consultation with an infectious diseases specialist is recommended. **(27)** Several species are intrinsically resistant to the lipopeptides (colistin and polymyxin B). Refer to Appendix B.										
Colistin or	–	–	–	–	–	–	–	≤ 2	≥ 4	**(28)** Colistin (methanesulfonate) should be given with a loading dose and maximum renally adjusted doses (see international consensus guidelines[9]). **(29)** Polymyxin B should be given with a loading dose and maximum recommended doses (see international consensus guidelines[9]). **(30)** When colistin or polymyxin B is given systemically, neither is likely to be effective for pneumonia. **(31)** For colistin, broth microdilution, CBDE, and CAT MIC methods are acceptable. For polymyxin B, broth microdilution is the only approved method. Disk diffusion and gradient diffusion methods should not be performed (see Table 3E).
polymyxin B*	–	–	–	–	–	–	–	≤ 2	≥ 4	
AMINOGLYCOSIDES										
(32) Breakpoints for gentamicin, tobramycin, and amikacin are based on population distributions of various species, PK/PD target attainment analyses with an end point of net bacterial stasis and limited clinical data. Clinical outcomes data for aminoglycosides as monotherapy for systemic infections are limited and have resulted in worse treatment outcomes (for infections outside of the urinary tract) compared with other therapies. Combination therapy for most indications other than UTIs should be considered. Consultation with an infectious diseases specialist is recommended.										
Gentamicin	10 µg	≥ 18	–	15–17^	≤ 14	≤ 2	–	4^	≥ 8	
Tobramycin	10 µg	≥ 17	–	13–16^	≤ 12	≤ 2	–	4^	≥ 8	
Amikacin	30 µg	≥ 20	–	17–19^	≤ 16	≤ 4	–	8^	≥ 16	

Table 2A-1. Enterobacterales (excluding *Salmonella* and *Shigella* spp.) (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm				Interpretive Categories and MIC Breakpoints, µg/mL				Comments
		S	SDD	I	R	S	SDD	I	R	
AMINOGLYCOSIDES (Continued)										
Plazomicin	30 µg	≥ 18	–	15–17^	≤ 14	≤ 2	–	4^	≥ 8	See comment (12).
Kanamycin*	30 µg	≥ 18	–	14–17^	≤ 13	≤ 16	–	32^	≥ 64	
Netilmicin*	30 µg	≥ 15	–	13–14^	≤ 12	≤ 8	–	16^	≥ 32	
Streptomycin*	10 µg	≥ 15	–	12–14^	≤ 11	–	–	–	–	
TETRACYCLINES										
(33) Isolates that **test** susceptible to tetracycline are considered susceptible to doxycycline and minocycline. **Isolates** that **test** intermediate or resistant to tetracycline **should be tested against doxycycline or minocycline if those results are needed for treatment.**										
Tetracycline	30 µg	≥ 15	–	12–14	≤ 11	≤ 4	–	8	≥ 16	
Doxycycline*	30 µg	≥ 14	–	11–13	≤ 10	≤ 4	–	8	≥ 16	
Minocycline*	30 µg	≥ 16	–	13–15	≤ 12	≤ 4	–	8	≥ 16	
QUINOLONES AND FLUOROQUINOLONES (Please refer to Glossary I.)										
Ciprofloxacin Levofloxacin	5 µg 5 µg	≥ 26 ≥ 21	– –	22–25^ 17–20^	≤ 21 ≤ 16	≤ 0.25 ≤ 0.5	– –	0.5^ 1^	≥ 1 ≥ 2	
Cinoxacin* (U)[a]	100 µg	≥ 19	–	15–18^	≤ 14	≤ 16	–	32^	≥ 64	
Enoxacin* (U)[a]	10 µg	≥ 18	–	15–17^	≤ 14	≤ 2	–	4^	≥ 8	
Gatifloxacin*	5 µg	≥ 18	–	15–17^	≤ 14	≤ 2	–	4^	≥ 8	
Gemifloxacin*	5 µg	≥ 20	–	16–19	≤ 15	≤ 0.25	–	0.5	≥ 1	**(34)** Report only on *K. pneumoniae.*
Grepafloxacin*	5 µg	≥ 18	–	15–17	≤ 14	≤ 1	–	2	≥ 4	
Lomefloxacin*	10 µg	≥ 22	–	19–21^	≤ 18	≤ 2	–	4^	≥ 8	
Nalidixic acid* (U)[a]	30 µg	≥ 19	–	14–18	≤ 13	≤ 16	–	–	≥ 32	
Norfloxacin* (U)[a]	10 µg	≥ 17	–	13–16	≤ 12	≤ 4	–	8	≥ 16	
Ofloxacin*	5 µg	≥ 16	–	13–15^	≤ 12	≤ 2	–	4^	≥ 8	
Fleroxacin (Inv.)	5 µg	≥ 19	–	16–18^	≤ 15	≤ 2	–	4^	≥ 8	

Table 2A-1. Enterobacterales (excluding *Salmonella* and *Shigella* spp.) (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm				Interpretive Categories and MIC Breakpoints, µg/mL				Comments
		S	SDD	I	R	S	SDD	I	R	
FOLATE PATHWAY ANTAGONISTS										
Trimethoprim-sulfamethoxazole	1.25/ 23.75 µg	≥ 16	–	11–15	≤ 10	≤ 2/38	–	–	≥ 4/76	
Sulfonamides* (U)[a]	250 or 300 µg	≥ 17	–	13–16	≤ 12	≤ 256	–	–	≥ 512	
Trimethoprim* (U)[a]	5 µg	≥ 16	–	11–15	≤ 10	≤ 8	–	–	≥ 16	
PHENICOLS										
Chloramphenicol*	30 µg	≥ 18	–	13–17	≤ 12	≤ 8	–	16	≥ 32	**(35)** Not routinely reported on isolates from the urinary tract.
FOSFOMYCINS										
Fosfomycin (U)[a]	200 µg	≥ 16	–	13–15	≤ 12	≤ 64	–	128	≥ 256	**(36)** Disk diffusion and MIC breakpoints apply only to *E. coli* urinary tract isolates and should not be extrapolated to other species of Enterobacterales. **(37)** The 200-µg fosfomycin disk contains 50 µg glucose-6-phosphate. **(38)** The only approved MIC method for testing is agar dilution using agar media supplemented with 25 µg/mL of glucose-6-phosphate. Broth dilution MIC testing should not be performed.
NITROFURANS										
Nitrofurantoin (U)[a]	300 µg	≥ 17	–	15–16	≤ 14	≤ 32	–	64	≥ 128	

Abbreviations: AST, antimicrobial susceptibility testing; CAMHB, cation-adjusted Mueller-Hinton broth; CAT, colistin agar test; CBDE, colistin broth disk elution; ESBL, extended-spectrum β-lactamase; I, intermediate; Inv., investigational agent; IV, intravenous; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; PK/PD, pharmacokinetic/pharmacodynamic; QC, quality control; R, resistant; S, susceptible; SDD, susceptible-dose dependent; U, urine; UTI, urinary tract infection.

Symbols: ^, designation for agents that have the potential to concentrate in the urine; *, designation for “Other” agents that are not included in Tables 1 but have established clinical breakpoints.

Table 2A-1. Enterobacterales (excluding *Salmonella* and *Shigella* spp.) (Continued)

Footnote

a. Report only on organisms isolated from the urinary tract.

References for Table 2A-1

[1] Hackel MA, Tsuji M, Yamano Y, Echols R, Karlowsky JA, Sahm DF. Reproducibility of broth microdilution MICs for the novel siderophore cephalosporin, cefiderocol, determined using iron-depleted cation-adjusted Mueller-Hinton broth. *Diagn Microbiol Infect Dis.* 2019;94(4):321-325. doi:10.1016/j.diagmicrobio.2019.03.003

[2] CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests.* 14th ed. CLSI standard M02. Clinical and Laboratory Standards Institute; 2024.

[3] CLSI. *M02 Disk Diffusion Reading Guide.* 2nd ed. CLSI quick guide M02-Ed14-QG. Clinical and Laboratory Standards Institute; 2024.

[4] Tamma PD, Aitken SL, Bonomo RA, Mathers AJ, van Duin D, Clancy CJ. IDSA 2024 guidance on the treatment of antimicrobial resistant gram-negative infections. Accessed 15 October 2024. https://www.idsociety.org/practice-guideline/amr-guidance/

[5] Perrott J, Mabasa VH, Ensom MHH. Comparing outcomes of meropenem administration strategies based on pharmacokinetic and pharmacodynamic principles: a qualitative systematic review. *Ann Pharmacother.* 2010;44(3):557-564. doi:10.1345/aph.1M339

[6] Cirillo I, Vaccaro N, Turner K, Solanki B, Natarajan J, Redman R. Pharmacokinetics, safety, and tolerability of doripenem after 0.5-, 1-, and 4-hour infusions in healthy volunteers. *J Clin Pharmacol.* 2009;49(7):798-806. doi:10.1177/0091270009337012

[7] Sakka SG, Glauner AK, Bulitta JB, et al. Population pharmacokinetics and pharmacodynamics of continuous versus short-term infusion of imipenem-cilastatin in critically ill patients in a randomized, controlled trial. *Antimicrob Agents Chemother.* 2007;51(9):3304-3310. doi:10.1128/AAC.01318-06

[8] Peleg AY, Hooper DC. Hospital-acquired infections due to gram-negative bacteria. *N Engl J Med.* 2010;362(19):1804-1813. doi:10.1056/NEJMra0904124

[9] Tsuji BT, Pogue JM, Zavascki AP, et al. International consensus guidelines for the optimal use of the polymyxins: endorsed by the American College of Clinical Pharmacy (ACCP), European Society of Clinical Microbiology and Infectious Diseases (ESCMID), Infectious Diseases Society of America (IDSA), International Society for Anti-Infective Pharmacology (ISAP), Society of Critical Care Medicine (SCCM), and Society of Infectious Diseases Pharmacists (SIDP). *Pharmacotherapy.* 2019;39(1):10-39. doi:10.1002/phar.2209

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Table 2A-2. Zone Diameter and MIC Breakpoints for *Salmonella* and *Shigella* spp.

Testing Conditions

Medium:	Disk diffusion: MHA Broth dilution: CAMHB Agar dilution: MHA
Inoculum:	Broth culture method or colony suspension, equivalent to a 0.5 McFarland standard; positive blood culture broth for select antimicrobial agents with disk diffusion (see general comment [5])
Incubation:	35°C ± 2°C; ambient air Disk diffusion: 16–18 hours Dilution methods: 16–20 hours

QC Recommendations

Refer to the following:

- **Tables 4A-1 and 5A-1 that list acceptable QC ranges applicable for each method**
- **Appendix I to develop a QC plan**

When a commercial test system is used for antimicrobial susceptibility testing, refer to the manufacturer's instructions for QC **strains** and QC ranges.

General Comments

(1) Refer to Table 1A-2 for antimicrobial agents that should be considered for testing and reporting by microbiology laboratories.

(2) For disk diffusion, test a maximum of 12 disks on a 150-mm plate and no more than 6 disks on a 100-mm plate; disks should be placed no less than 24 mm apart, center to center (see CLSI M02)[1]. Each zone diameter should be clearly measurable; overlapping zones prevent accurate measurement. Measure the diameter of the zones of complete inhibition (as judged by the unaided eye), including the diameter of the disk (see CLSI M02QG[2]). Hold the Petri plate a few inches above a black background illuminated with reflected light. The zone margin should be considered the area showing no obvious, visible growth that can be detected with the unaided eye. Ignore faint growth of tiny colonies that can be detected only with a magnifying lens at the edge of the zone of inhibited growth. With trimethoprim-sulfamethoxazole, antagonists in the medium may allow some slight growth; therefore, disregard slight growth (20% or less of the lawn of growth) and measure the more obvious margin to determine the zone diameter.

(3) When fecal isolates of *Salmonella* and *Shigella* spp. are tested, only ampicillin, a fluoroquinolone, and trimethoprim-sulfamethoxazole should be reported routinely. Data regarding whether amoxicillin should be used to treat shigellosis are conflicting. When reporting ampicillin results, state that treatment of shigellosis with amoxicillin might have poorer efficacy compared with treatment with ampicillin. In addition, for extraintestinal isolates of *Salmonella* spp., a third-generation cephalosporin should be tested and reported, and chloramphenicol may be tested and reported if requested. Susceptibility testing is indicated for typhoidal *Salmonella* (*S. enterica* ser. Typhi and *S. enterica* ser. Paratyphi A–C) isolated from extraintestinal and intestinal sources. Routine susceptibility testing is not indicated for nontyphoidal *Salmonella* spp. isolated from intestinal sources. In contrast, susceptibility testing is indicated for all *Shigella* isolates.

Table 2A-2. *Salmonella* and *Shigella* spp. (Continued)

(4) An intermediate (I) with a ^ in Tables 2 indicates agents that have the potential to concentrate in the urine. The I^ is for informational use only. The decision to report I^ is best made by each laboratory based on institution-specific guidelines and in consultation with appropriate medical personnel.

(5) Positive blood culture broth can be used as the inoculum for direct disk diffusion testing of select antimicrobial agents against Enterobacterales (using methods described in Table 3F-1 and applying breakpoints in Table 3F-2). Only drugs appropriate for *Salmonella* or *Shigella* spp. should be reported. For antimicrobial agents not listed in Table 3F-2 for Enterobacterales, CLSI has not yet evaluated this direct disk diffusion method.

NOTE: Information in boldface type is new or modified since the previous edition.

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm				Interpretive Categories and MIC Breakpoints, µg/mL				Comments
		S	SDD	I	R	S	SDD	I	R	
PENICILLINS										
Ampicillin	10 µg	≥ 17	–	14–16^	≤ 13	≤ 8	–	16^	≥ 32	**(6)** Results of ampicillin testing can be used to predict results for amoxicillin. **(7)** Breakpoints when oral ampicillin is used for therapy of salmonellosis or shigellosis. See general comment (3).
CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.)										
(8) WARNING: First- and second-generation cephalosporins and cephamycins may appear active *in vitro* but are not effective clinically and should not be reported as susceptible.										
Cefotaxime or	30 µg	≥ 26	–	23–25^	≤ 22	≤ 1	–	2^	≥ 4	
ceftriaxone	30 µg	≥ 23		20–22^	≤ 19	≤ 1		2^	≥ 4	
CARBAPENEMS										
(9) Ertapenem, imipenem, and/or meropenem might be considered for testing for isolates resistant to all other agents listed in Table 1A-2, although there are limited clinical data suggesting their effectiveness for treating salmonellosis or shigellosis.[3]										
Ertapenem	10 µg	≥ 22	–	19–21^	≤ 18	≤ 0.5	–	1^	≥ 2	
Imipenem	10 µg	≥ 23	–	20–22^	≤ 19	≤ 1	–	2^	≥ 4	
Meropenem	10 µg	≥ 23	–	20–22^	≤ 19	≤ 1	–	2^	≥ 4	

Table 2A-2. *Salmonella* and *Shigella* spp. (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm				Interpretive Categories and MIC Breakpoints, µg/mL				Comments
		S	SDD	I	R	S	SDD	I	R	
MACROLIDES										
Azithromycin	15 µg	≥ 13	–	–	≤ 12	≤ 16	–	–	≥ 32	**(10)** *S. enterica* ser. Typhi only: breakpoints are based on MIC distribution data and limited clinical data.
		≥ 16	–	11–15	≤ 10	≤ 8	–	16	≥ 32	**(11)** *Shigella* spp. only: azithromycin disk diffusion zones can be hazy and difficult to measure, especially *Shigella sonnei*. If an isolate has a zone of inhibition that is difficult to measure, an MIC method is recommended. Media source may affect the clarity of the end points for disk diffusion tests.
TETRACYCLINES										
(12) Isolates that **test** susceptible to tetracycline are considered susceptible to doxycycline and minocycline. **Isolates** that **test** intermediate or resistant to tetracycline **should be tested against doxycycline or minocycline if those results are needed for treatment.**										
Tetracycline	30 µg	≥ 15	–	12–14	≤ 11	≤ 4	–	8	≥ 16	
Doxycycline*	30 µg	≥ 14	–	11–13	≤ 10	≤ 4	–	8	≥ 16	
Minocycline*	30 µg	≥ 16	–	13–15	≤ 12	≤ 4	–	8	≥ 16	
FLUOROQUINOLONES for *Salmonella* spp.										
(13) For testing and reporting of *Salmonella* spp. (including *S. enterica* ser. Typhi and *S. enterica* ser. Paratyphi A-C). Routine susceptibility testing is not indicated for nontyphoidal *Salmonella* spp. isolated from intestinal sources. **(14)** The preferred test for assessing fluoroquinolone susceptibility or resistance in *Salmonella* spp. is a ciprofloxacin MIC test. A levofloxacin or ofloxacin MIC test can be performed if either agent, respectively, is the fluoroquinolone of choice in a specific facility. If a ciprofloxacin, levofloxacin, or ofloxacin MIC or ciprofloxacin disk diffusion test cannot be done, pefloxacin disk diffusion may be used as a surrogate test to predict ciprofloxacin susceptibility. **(15)** No single test detects resistance resulting from all possible fluoroquinolone resistance mechanisms that have been identified in *Salmonella* spp.										
Ciprofloxacin	5 µg	≥ 31	–	21–30^	≤ 20	≤ 0.06	–	0.12–0.5^	≥ 1	**(16)** Isolates of *Salmonella* spp. that test not susceptible to ciprofloxacin, levofloxacin, ofloxacin, or pefloxacin may be associated with clinical failure or delayed response in fluoroquinolone-treated patients with salmonellosis.
Levofloxacin	–	–	–	–	–	≤ 0.12	–	0.25–1^	≥ 2	
Ofloxacin*	–	–	–	–	–	≤ 0.12	–	0.25–1^	≥ 2	

Table 2A-2. *Salmonella* and *Shigella* spp. (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm				Interpretive Categories and MIC Breakpoints, µg/mL				Comments
		S	SDD	I	R	S	SDD	I	R	
FLUOROQUINOLONES for *Salmonella* spp. (Continued)										
Pefloxacin (Inv.) (surrogate test for ciprofloxacin)	5 µg	≥ 24	–	–	≤ 23	–	–	–	–	**(17)** Report results as ciprofloxacin susceptible or resistant based on the pefloxacin result. Pefloxacin will not detect resistance in *Salmonella* spp. due to *aac(6')-Ib-cr.* Pefloxacin disks are not available in the United States. See comment (15).
FLUOROQUINOLONES for *Shigella* spp.										
Ciprofloxacin	5 µg	≥ 26	–	22–25^	≤ 21	≤ 0.25	–	0.5^	≥ 1	
Levofloxacin	5 µg	≥ 21	–	17–20^	≤ 16	≤ 0.5	–	1^	≥ 2	
Ofloxacin*	5 µg	≥ 16	–	13–15^	≤ 12	≤ 2	–	4^	≥ 8	
FOLATE PATHWAY ANTAGONISTS										
Trimethoprim-sulfamethoxazole	1.25/23.75 µg	≥ 16	–	11–15	≤ 10	≤ 2/38	–	–	≥ 4/76	See general comment (3).
PHENICOLS										
Chloramphenicol*	30 µg	≥ 18	–	13–17	≤ 12	≤ 8	–	16	≥ 32	**(18)** Not routinely reported on isolates from the urinary tract.

Abbreviations: CAMHB, cation-adjusted Mueller-Hinton broth; I, intermediate; Inv., investigational agent; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; QC, quality control; R, resistant; S, susceptible; SDD, susceptible-dose dependent.

Symbols: ^, designation for agents that have the potential to concentrate in the urine; *, designation for "Other" agents that are not included in Tables 1 but have established clinical breakpoints.

References for Table 2A-2

[1] CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests.* 14th ed. CLSI standard M02. Clinical and Laboratory Standards Institute; 2024.

[2] CLSI. *M02 Disk Diffusion Reading Guide.* 2nd ed. CLSI quick guide M02-Ed14-QG. Clinical and Laboratory Standards Institute; 2024.

[3] CDC Health Alert Network. Extensively drug-resistant *Salmonella* Typhi infections among U.S. residents without international travel. Accessed 15 October 2024. https://emergency.cdc.gov/han/pdf/CDC-HAN-439-XDR-Salmonella-Typhi-Infections-in-U.S.-Without-Intl-Travel-02.12.2021.pdf

Table 2B-1. Zone Diameter and MIC Breakpoints for *Pseudomonas aeruginosa*

Testing Conditions

Medium:	Disk diffusion: MHA Broth dilution: CAMHB; iron-depleted CAMHB for cefiderocol (see Appendix H, section H1)[1] Agar dilution: MHA
Inoculum:	Broth culture method or colony suspension, equivalent to a 0.5 McFarland standard; positive blood culture broth for select antimicrobial agents with disk diffusion (see general comment [6])
Incubation:	35°C ± 2°C; ambient air Disk diffusion: 16–18 hours Dilution methods: 16–20 hours

QC Recommendations

Refer to the following:

- **Tables 4A-1, 4A-2, 5A-1, and 5A-2 that list acceptable QC ranges applicable for each method**
- **Appendix I to develop a QC plan**

When a commercial test system is used for antimicrobial susceptibility testing, refer to the manufacturer's instructions for QC **strains** and QC ranges.

Refer to Tables 3B, 3C, 3E, 3F-1, and 3F-3 for additional testing recommendations, reporting suggestions, and QC.

General Comments

(1) Refer to Table 1B-1 for antimicrobial agents that should be considered for testing and reporting by microbiology laboratories.

(2) For disk diffusion, test a maximum of 12 disks on a 150-mm plate and no more than 6 disks on a 100-mm plate; disks should be placed no less than 24 mm apart, center to center (see CLSI M02[2]). Each zone diameter should be clearly measurable; overlapping zones prevent accurate measurement. Measure the diameter of the zones of complete inhibition (as judged by the unaided eye), including the diameter of the disk (see CLSI M02QG[3]). Hold the Petri plate a few inches above a black background illuminated with reflected light. The zone margin should be considered the area showing no obvious, visible growth that can be detected with the unaided eye. Ignore faint growth of tiny colonies that can be detected only with a magnifying lens at the edge of the zone of inhibited growth.

(3) The susceptibility of *P. aeruginosa* isolated from patients with cystic fibrosis can be reliably determined by disk diffusion or dilution methods but may need extended incubation for up to 24 hours before reporting as susceptible.

(4) *P. aeruginosa* may develop resistance during therapy with all antimicrobial agents. Therefore, isolates that are initially susceptible may become resistant within a few days after initiation of therapy. Testing of repeat isolates may be warranted.

(5) An intermediate (I) with a ^ in Tables 2 indicates agents that have the potential to concentrate in the urine. The I^ is for informational use only. The decision to report I^ is best made by each laboratory based on institution-specific guidelines and in consultation with appropriate medical personnel.

Table 2B-1. *Pseudomonas aeruginosa* (Continued)

(6) Positive blood culture broth can be used as the inoculum for direct disk diffusion testing of select antimicrobial agents against *P. aeruginosa* (using methods described in Table 3F-1 and applying breakpoints in Table 3F-3). For antimicrobial agents not listed in Table 3F-3 for *P. aeruginosa*, CLSI has not yet evaluated this direct disk diffusion method.

NOTE: Information in boldface type is new or modified since the previous edition.

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
		S	I	R	S	I	R	
PENICILLINS								
Piperacillin*	100 µg	≥ 22	18–21^	≤ 17	≤ 16	32^	≥ 64	
β-LACTAM COMBINATION AGENTS								
(7) Organisms that test susceptible to the β-lactam agent alone are also considered susceptible to the β-lactam combination agent. However, organisms that test susceptible to the β-lactam combination agent cannot be assumed to be susceptible to the β-lactam agent alone. Similarly, organisms that test intermediate or resistant to the β-lactam agent alone may be susceptible to the β-lactam combination agent.								
Piperacillin-tazobactam	100/10 µg	≥ 22	18–21	≤ 17	≤ 16/4	32/4	≥ 64/4	**(8)** Breakpoints for intermediate are only to provide a buffer zone to prevent small uncontrolled technical factors from causing major discrepancies in interpretation.
Ceftazidime-avibactam	30/20 µg	≥ 21	–	≤ 20	≤ 8/4	–	≥ 16/4	
Ceftolozane-tazobactam	30/10 µg	≥ 21	17–20^	≤ 16	≤ 4/4	8/4^	≥ 16/4	
Imipenem-relebactam	10/25 µg	≥ 23	20–22^	≤ 19	≤ 2/4	4/4^	≥ 8/4	
Ticarcillin-clavulanate*	75/10 µg	≥ 24	16–23^	≤ 15	≤ 16/2	32/2–64/2^	≥ 128/2	
CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.)								
Ceftazidime	30 µg	≥ 18	15–17^	≤ 14	≤ 8	16^	≥ 32	
Cefepime	30 µg	≥ 18	15–17^	≤ 14	≤ 8	16^	≥ 32	

Table 2B-1. *Pseudomonas aeruginosa* (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
		S	I	R	S	I	R	
CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.) (Continued)								
Cefiderocol	30 µg	≥ 18	13–17^	≤ 12	≤ 4	8^	≥ 16	**(9)** The accuracy and reproducibility of cefiderocol testing results by disk diffusion and broth microdilution are markedly affected by iron concentration and inoculum preparation and may vary by disk and media manufacturer. Depending on the type of variance observed, false-resistant or false-susceptible results may occur. Testing subsequent isolates is encouraged. Discussion with prescribers and antimicrobial stewardship members regarding the potential for inaccuracies is recommended.
MONOBACTAMS								
Aztreonam	30 µg	≥ 22	16–21^	≤ 15	≤ 8	16^	≥ 32	
CARBAPENEMS								
Doripenem*	10 µg	≥ 19	16–18^	≤ 15	≤ 2	4^	≥ 8	
Imipenem	10 µg	≥ 19	16–18^	≤ 15	≤ 2	4^	≥ 8	
Meropenem	10 µg	≥ 19	16–18^	≤ 15	≤ 2	4^	≥ 8	

Table 2B-1. *Pseudomonas aeruginosa* (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, μg/mL			Comments
		S	I	R	S	I	R	
LIPOPEPTIDES								
(10) WARNING: Clinical and PK/PD data demonstrate colistin and polymyxin B have limited clinical efficacy, even if an intermediate result is obtained. Alternative agents are strongly preferred. Colistin and polymyxin B should be used in combination with one or more active antimicrobial agents. Consultation with an infectious diseases specialist is recommended.								
Colistin or polymyxin B*	– –	– –	– –	– –	– –	≤ 2 ≤ 2	≥ 4 ≥ 4	**(11)** Colistin (methanesulfonate) should be given with a loading dose and maximum renally adjusted doses (see international consensus guidelines[4]). **(12)** Polymyxin B should be given with a loading dose and maximum recommended doses (see international consensus guidelines[4]). **(13)** When colistin or polymyxin B is given systemically, neither is likely to be effective for pneumonia. **(14)** For colistin, broth microdilution, CBDE, and CAT MIC methods are acceptable. For polymyxin B, broth microdilution is the only approved method. Disk diffusion and gradient diffusion methods should not be performed (see Table 3E).
AMINOGLYCOSIDES								
(15) Breakpoints for tobramycin and amikacin are based on population distributions of various species, PK/PD target attainment analyses with an end point of net bacterial stasis, and limited clinical data. Clinical outcomes data for aminoglycosides as monotherapy for systemic infections are limited and have resulted in worse treatment outcomes (for infections outside of the urinary tract) compared with other therapies. Combination therapy for most indications other than UTIs should be considered. Consultation with an infectious diseases specialist is recommended.								
Tobramycin	10 μg	≥ 19	13–18^	≤ 12	≤ 1	2^	≥ 4	**(16)** Tobramycin does not predict susceptibility to gentamicin.
Amikacin (U)[a]	30 μg	≥ 17	15–16^	≤ 14	≤ 16	32^	≥ 64	
Netilmicin*	30 μg	≥ 15	13–14^	≤ 12	≤ 8	16^	≥ 32	
FLUOROQUINOLONES								
Ciprofloxacin	5 μg	≥ 25	19–24^	≤ 18	≤ 0.5	1^	≥ 2	
Levofloxacin	5 μg	≥ 22	15–21^	≤ 14	≤ 1	2^	≥ 4	

Table 2B-1. *Pseudomonas aeruginosa* (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
		S	I	R	S	I	R	
FLUOROQUINOLONES (Continued)								
Lomefloxacin* (U)[a]	10 µg	≥ 22	19–21^	≤ 18	≤ 2	4^	≥ 8	
Norfloxacin* (U)[a]	10 µg	≥ 17	13–16	≤ 12	≤ 4	8	≥ 16	
Ofloxacin*	5 µg	≥ 16	13–15^	≤ 12	≤ 2	4^	≥ 8	
Gatifloxacin*	5 µg	≥ 18	15–17^	≤ 14	≤ 2	4^	≥ 8	

Abbreviations: CAMHB, cation-adjusted Mueller-Hinton broth; CAT, colistin agar test; CBDE, colistin broth disk elution; I, intermediate; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; PK/PD, pharmacokinetic/pharmacodynamic; QC, quality control; R, resistant; S, susceptible; U, urine; UTI, urinary tract infection.
Symbols: ^, designation for agents that have the potential to concentrate in the urine; *, designation for “Other” agents that are not included in Tables 1 but have established clinical breakpoints.

Footnote

a. Report only on organisms isolated from the urinary tract.

References for Table 2B-1

[1] Hackel MA, Tsuji M, Yamano Y, Echols R, Karlowsky JA, Sahm DF. Reproducibility of broth microdilution MICs for the novel siderophore cephalosporin, cefiderocol, determined using iron-depleted cation-adjusted Mueller-Hinton broth. *Diagn Microbiol Infect Dis.* 2019;94(4):321-325. doi:10.1016/j.diagmicrobio.2019.03.003

[2] CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests.* 14th ed. CLSI standard M02. Clinical and Laboratory Standards Institute; 2024.

[3] CLSI. *M02 Disk Diffusion Reading Guide.* 2nd ed. CLSI quick guide M02-Ed14-QG. Clinical and Laboratory Standards Institute; 2024.

[4] Tsuji BT, Pogue JM, Zavascki AP, et al. International consensus guidelines for the optimal use of the polymyxins: endorsed by the American College of Clinical Pharmacy (ACCP), European Society of Clinical Microbiology and Infectious Diseases (ESCMID), Infectious Diseases Society of America (IDSA), International Society for Anti-Infective Pharmacology (ISAP), Society of Critical Care Medicine (SCCM), and Society of Infectious Diseases Pharmacists (SIDP). *Pharmacotherapy.* 2019;39(1):10-39. doi:10.1002/phar.2209

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Table 2B-2. Zone Diameter and MIC Breakpoints for *Acinetobacter* spp.

Testing Conditions

Medium: Disk diffusion: MHA
Broth dilution: CAMHB; iron-depleted CAMHB for cefiderocol (see Appendix H, section H1)[1]
Agar dilution: MHA

Inoculum: Broth culture method or colony suspension, equivalent to a 0.5 McFarland standard; positive blood culture broth for select antimicrobial agents with disk diffusion (see general comment [3])

Incubation: 35°C ± 2°C; ambient air; 20–24 hours, all methods

QC Recommendations

Refer to the following:

- **Tables 4A-1, 4A-2, 5A-1, and 5A-2 that list acceptable QC ranges applicable for each method**
- **Appendix I to develop a QC plan**

When a commercial test system is used for antimicrobial susceptibility testing, refer to the manufacturer's instructions for QC **strains** and QC ranges.

General Comments

(1) Refer to Table 1B-2 for antimicrobial agents that should be considered for testing and reporting by microbiology laboratories.

(2) For disk diffusion, test a maximum of 12 disks on a 150-mm plate and no more than 6 disks on a 100-mm plate; disks should be placed no less than 24 mm apart, center to center (see CLSI M02[2]). Each zone diameter should be clearly measurable; overlapping zones prevent accurate measurement. Measure the diameter of the zones of complete inhibition (as judged by the unaided eye), including the diameter of the disk (see CLSI M02QG[3]). Hold the Petri plate a few inches above a black background illuminated with reflected light. The zone margin should be considered the area showing no obvious, visible growth that can be detected with the unaided eye. Ignore faint growth of tiny colonies that can be detected only with a magnifying lens at the edge of the zone of inhibited growth. With trimethoprim and the sulfonamides, antagonists in the medium may allow some slight growth; therefore, disregard slight growth (20% or less of the lawn of growth) and measure the more obvious margin to determine the zone diameter.

(3) Positive blood culture broth can be used as the inoculum for direct disk diffusion testing of select antimicrobial agents against *Acinetobacter* spp. (using methods described in Table 3F-1 and applying breakpoints in Table 3F-4). For antimicrobial agents not listed in Table 3F-4 for *Acinetobacter* spp., CLSI has not yet evaluated this direct disk diffusion method.

NOTE: Information in boldface type is new or modified since the previous edition.

Table 2B-2. *Acinetobacter* spp. (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
		S	I	R	S	I	R	
PENICILLINS								
Piperacillin*	100 µg	≥ 21	18–20	≤ 17	≤ 16	32–64	≥ 128	
β-LACTAM COMBINATION AGENTS								
(4) Organisms that test susceptible to the β-lactam agent alone are also considered susceptible to the β-lactam combination agent. However, organisms that test susceptible to the β-lactam combination agent cannot be assumed to be susceptible to the β-lactam agent alone. Similarly, organisms that test intermediate or resistant to the β-lactam agent alone may be susceptible to the β-lactam combination agent.								
Ampicillin-sulbactam	10/10 µg	**≥ 22**	**17–21**	**≤ 16**	≤ 8/4	16/8	≥ 32/16	
Piperacillin-tazobactam	100/10 µg	≥ 21	18–20	≤ 17	≤ 16/4	32/4–64/4	≥ 128/4	
Sulbactam-durlobactam	10/10 µg	≥ 17	14–16	≤ 13	≤ 4/4	8/4	≥ 16/4	
Ticarcillin-clavulanate*	75/10 µg	≥ 20	15–19	≤ 14	≤ 16/2	32/2–64/2	≥ 128/2	
CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.)								
Ceftazidime	30 µg	≥ 18	15–17	≤ 14	≤ 8	16	≥ 32	
Cefepime	30 µg	≥ 18	15–17	≤ 14	≤ 8	16	≥ 32	
Cefotaxime	30 µg	≥ 23	15–22	≤ 14	≤ 8	16–32	≥ 64	
Ceftriaxone	30 µg	≥ 21	14–20	≤ 13	≤ 8	16–32	≥ 64	

Table 2B-2. *Acinetobacter* spp. (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
		S	I	R	S	I	R	
CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.) (Continued)								
Cefiderocol	30 µg	≥ 15	–	–	≤ 4	8	≥ 16	**(5)** Disk diffusion zone diameters ≤ 14 mm should not be interpreted or reported because zone diameters ≤ 14 mm occur with resistant, intermediate, and susceptible isolates. For isolates with zone diameters ≤ 14 mm, do not report cefiderocol without performing an MIC test. **(6)** Report only on *A. baumannii* complex. **(7)** The accuracy and reproducibility of cefiderocol testing results by disk diffusion and broth microdilution are markedly affected by iron concentration and inoculum preparation and may vary by disk and media manufacturer. Depending on the type of variance observed, false-resistant or false-susceptible results may occur. Testing subsequent isolates is encouraged. Discussion with prescribers and antimicrobial stewardship members regarding the potential for inaccuracies is recommended.
CARBAPENEMS								
Doripenem*	10 µg	≥ 18	15–17	≤ 14	≤ 2	4	≥ 8	
Imipenem	10 µg	≥ 22	19–21	≤ 18	≤ 2	4	≥ 8	
Meropenem	10 µg	≥ 18	15–17	≤ 14	≤ 2	4	≥ 8	
LIPOPEPTIDES								
(8) WARNING: Clinical and PK/PD data demonstrate colistin and polymyxin B have limited clinical efficacy, even if an intermediate result is obtained. Alternative agents are strongly preferred. Colistin and polymyxin B should be used in combination with one or more active antimicrobial agents. Consultation with an infectious diseases specialist is recommended.								

Table 2B-2. *Acinetobacter* spp. (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
		S	I	R	S	I	R	
LIPOPEPTIDES (Continued)								
Colistin or	–	–	–	–	–	≤ 2	≥ 4	**(9)** Colistin (methanesulfonate) should be given with a loading dose and maximum renally adjusted doses (see international consensus guidelines[4]). **(10)** Polymyxin B should be given with a loading dose and maximum recommended doses (see international consensus guidelines[4]). **(11)** When colistin or polymyxin B is given systemically, the drug is unlikely to be effective for pneumonia. **(12)** The only approved MIC method is broth microdilution. CBDE, CAT, disk diffusion, and gradient diffusion should not be performed. See comment (6).
polymyxin B	–	–	–	–	–	≤ 2	≥ 4	
AMINOGLYCOSIDES								
Gentamicin	10 µg	≥ 15	13–14	≤ 12	≤ 4	8	≥ 16	
Tobramycin	10 µg	≥ 15	13–14	≤ 12	≤ 4	8	≥ 16	
Amikacin	30 µg	≥ 17	15–16	≤ 14	≤ 16	32	≥ 64	
Netilmicin*	–	–	–	–	≤ 8	16	≥ 32	
TETRACYCLINES								
Minocycline	30 µg	**≥ 22**	**18–21**	**≤ 17**	**≤ 1**	**2**	**≥ 4**	**(13) If needed for treatment, confirmatory MIC testing is indicated for isolates with zones of 18–21 mm to avoid reporting false-intermediate results.**
FLUOROQUINOLONES								
Ciprofloxacin	5 µg	≥ 21	16–20	≤ 15	≤ 1	2	≥ 4	
Levofloxacin	5 µg	≥ 17	14–16	≤ 13	≤ 2	4	≥ 8	
Gatifloxacin*	5 µg	≥ 18	15–17	≤ 14	≤ 2	4	≥ 8	

Table 2B-2. *Acinetobacter* spp. (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
		S	I	R	S	I	R	
FOLATE PATHWAY ANTAGONISTS								
Trimethoprim-sulfamethoxazole	1.25/23.75 µg	≥ 16	11–15	≤ 10	≤ 2/38	–	≥ 4/76	

Abbreviations: CAMHB, cation-adjusted Mueller-Hinton broth; CAT, colistin agar test; CBDE, colistin broth elution test; I, intermediate; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; PK/PD, pharmacokinetic/pharmacodynamic; QC, quality control; R, resistant; S, susceptible.
Symbol: *, designation for "Other" agents that are not included in Tables 1 but have established clinical breakpoints.

References for Table 2B-2

[1] Hackel MA, Tsuji M, Yamano Y, Echols R, Karlowsky JA, Sahm DF. Reproducibility of broth microdilution MICs for the novel siderophore cephalosporin, cefiderocol, determined using iron-depleted cation-adjusted Mueller-Hinton broth. *Diagn Microbiol Infect Dis.* 2019;94(4):321-325. doi:10.1016/j.diagmicrobio.2019.03.003

[2] CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests.* 14th ed. CLSI standard M02. Clinical and Laboratory Standards Institute; 2024.

[3] CLSI. *M02 Disk Diffusion Reading Guide.* 2nd ed. CLSI quick guide M02-Ed14-QG. Clinical and Laboratory Standards Institute; 2024.

[4] Tsuji BT, Pogue JM, Zavascki AP, et al. International consensus guidelines for the optimal use of the polymyxins: endorsed by the American College of Clinical Pharmacy (ACCP), European Society of Clinical Microbiology and Infectious Diseases (ESCMID), Infectious Diseases Society of America (IDSA), International Society for Anti-Infective Pharmacology (ISAP), Society of Critical Care Medicine (SCCM), and Society of Infectious Diseases Pharmacists (SIDP). *Pharmacotherapy.* 2019;39(1):10-39. doi:10.1002/phar.2209

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Table 2B-3. MIC Breakpoints for *Burkholderia cepacia* Complex

Testing Conditions

Medium: Broth dilution: CAMHB

Inoculum: Broth culture method or colony suspension, equivalent to a 0.5 McFarland standard

Incubation: 35°C ± 2°C; ambient air; 20–24 hours

QC Recommendations

Refer to the following:

- **Table 5A-1 that lists acceptable QC ranges**
- **Appendix I to develop a QC plan**

General Comments

(1) **Minimal inhibitory concentration (MIC) and disk diffusion breakpoints for *B. cepacia* complex organisms were removed based on data showing that two CLSI reference antimicrobial susceptibility testing (AST) methods, broth microdilution (BMD) and agar dilution, do not correlate. These findings are supported by additional studies conducted by European Committee on Antimicrobial Susceptibility Testing (EUCAST) and a Brazilian study demonstrating problems with *B. cepacia* complex AST.[1,2]**

(2) **Epidemiological cutoff values (ECVs) are available in Appendix F, which are for epidemiological use only. In several cases, ECVs are above MICs typically achievable by routine antimicrobial dosing for similar organisms.**

(3) **Laboratories can consider adding the following comment to the laboratory report: “Antimicrobial susceptibility testing is not routinely performed for *B. cepacia* complex due to the lack of accurate test methods. MICs for ceftazidime, levofloxacin, meropenem, minocycline, or trimethoprim-sulfamethoxazole with wild-type isolates are high and might be above the MICs typically achievable by routine antimicrobial dosing.”**

(4) **If testing is performed, reference BMD (frozen) is the only reproducible method and laboratories might consider including the comment, “correlation of MIC values with clinical outcome is not known.”**

NOTE: Information in boldface type is new or modified since the previous edition.

References for Table 2B-3

[1] Wootton M, Davies L, Pitman K, Howe RA. Evaluation of susceptibility testing methods for *Burkholderia cepacia* complex: a comparison of broth microdilution, agar dilution, gradient strip and EUCAST disc diffusion. *Clin Microbiol Infect*. 2020; S1198-743X(20)30708-4. doi:10.1016/j.cmi.2020.11.012

[2] Fehlberg LCC, Nicoletti AG, Ramos AC, et al. *In vitro* susceptibility of *Burkholderia cepacia* complex isolates: comparison of disk diffusion, Etest®, agar dilution, and broth microdilution methods. *Diagn Microbiol Infect Dis*. 2016; 86(4):422-427. doi:10.1016/j.diagmicrobio.2016.08.015

Table 2B-4. Zone Diameter and MIC Breakpoints for *Stenotrophomonas maltophilia*

Testing Conditions

Medium: Disk diffusion: MHA
Broth dilution: CAMHB; iron-depleted CAMHB for cefiderocol (see Appendix H, section H1)[1]
Agar dilution: MHA

Inoculum: Broth culture method or colony suspension, equivalent to a 0.5 McFarland standard

Incubation: 35°C ± 2°C; ambient air; 20–24 hours, all methods

QC Recommendations

Refer to the following:

- **Tables 4A-1, 5A-1, and 5A-2 that list acceptable QC ranges applicable for each method**
- **Appendix I to develop a QC plan**

When a commercial test system is used for antimicrobial susceptibility testing, refer to the manufacturer's instructions for QC **strains** and QC ranges.

Refer to Table 3D for additional testing recommendations, reporting suggestions, and QC.

General Comments

(1) Refer to Table 1B-4 for antimicrobial agents that should be considered for testing and reporting by microbiology laboratories.

(2) For disk diffusion, test a maximum of 12 disks on a 150-mm plate and no more than 6 disks on a 100-mm plate; disks should be placed no less than 24 mm apart, center to center (see CLSI M02[2]). Each zone diameter should be clearly measurable; overlapping zones prevent accurate measurement. Measure the diameter of the zones of complete inhibition (as judged by the unaided eye), including the diameter of the disk (see CLSI M02QG[3]). Hold the Petri plate a few inches above a black background illuminated with reflected light. The zone margin should be considered the area showing no obvious, visible growth that can be detected with the unaided eye. Ignore faint growth of tiny colonies that can be detected only with a magnifying lens at the edge of the zone of inhibited growth. With trimethoprim and the sulfonamides, antagonists in the medium may allow some slight growth; therefore, disregard slight growth (20% or less of the lawn of growth) and measure the more obvious margin to determine the zone diameter.

NOTE: Information in boldface type is new or modified since the previous edition.

Table 2B-4. *Stenotrophomonas maltophilia* (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
		S	I	R	S	I	R	
β-LACTAM COMBINATION AGENTS								
Ticarcillin-clavulanate*	–	–	–	–	≤ 16/2	32/2–64/2	≥ 128/2	
CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.)								
Cefiderocol	30 µg	≥ 15	–	–	≤ 1	–	–	**(3)** Breakpoints are based on PK/PD properties, MIC distributions, and limited clinical data. **(4)** The accuracy and reproducibility of cefiderocol testing results by disk diffusion and broth microdilution are markedly affected by iron concentration and inoculum preparation and may vary by disk and media manufacturer. Depending on the type of variance observed, false-resistant or false-susceptible results may occur. Testing subsequent isolates is encouraged. Discussion with prescribers and antimicrobial stewardship members regarding the potential for inaccuracies is recommended.
TETRACYCLINES								
Minocycline	30 µg	≥ 26	21–25	≤ 20	≤ 1	2	≥ 4	
FLUOROQUINOLONES								
Levofloxacin	5 µg	≥ 17	14–16	≤ 13	≤ 2	4	≥ 8	**(5)** ***Rx:*** Levofloxacin should not be used alone for antimicrobial therapy.
FOLATE PATHWAY ANTAGONISTS								
Trimethoprim-sulfamethoxazole	1.25/23.75 µg	≥ 16	11–15	≤ 10	≤ 2/38	–	≥ 4/76	**(6)** ***Rx:*** Trimethoprim-sulfamethoxazole should not be used alone for antimicrobial therapy.
PHENICOLS								
Chloramphenicol*	–	–	–	–	≤ 8	16	≥ 32	**(7)** Not routinely reported on organisms isolated from the urinary tract.

Abbreviations: CAMHB, cation-adjusted Mueller-Hinton broth; I, intermediate; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; PK/PD, pharmacokinetic/pharmacodynamic; QC, quality control; R, resistant; S, susceptible.
Symbol: *, designation for "Other" agents that are not included in Tables 1 but have established clinical breakpoints.

Table 2B-4. *Stenotrophomonas maltophilia* (Continued)

References for Table 2B-4

[1] Hackel MA, Tsuji M, Yamano Y, Echols R, Karlowsky JA, Sahm DF. Reproducibility of broth microdilution MICs for the novel siderophore cephalosporin, cefiderocol, determined using iron-depleted cation-adjusted Mueller-Hinton broth. *Diagn Microbiol Infect Dis*. 2019;94(4):321-325. doi:10.1016/j.diagmicrobio.2019.03.003

[2] CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests*. 14th ed. CLSI standard M02. Clinical and Laboratory Standards Institute; 2024.

[3] CLSI. *M02 Disk Diffusion Reading Guide*. 2nd ed. CLSI quick guide M02QG. Clinical and Laboratory Standards Institute; 2024.

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Table 2B-5. MIC Breakpoints for Other Non-Enterobacterales (Refer to General Comment [2])

Testing Conditions	QC Recommendations
Medium: Broth dilution: CAMHB Agar dilution: MHA **Inoculum:** Broth culture method or colony suspension, equivalent to a 0.5 McFarland standard **Incubation:** 35°C ± 2°C; ambient air; 16–20 hours	**Refer to the following:** • **Tables 5A-1 and 5A-2 that list acceptable QC ranges applicable for each method** • **Appendix I to develop a QC plan** When a commercial test system is used for antimicrobial susceptibility testing, refer to the manufacturer's instructions for QC **strains** and QC ranges.

General Comments

(1) Refer to Table 1B-5 for antimicrobial agents that should be considered for testing and reporting by microbiology laboratories.

(2) Other non-Enterobacterales include *Pseudomonas* spp. and other nonfastidious, glucose-nonfermenting, gram-negative bacilli but exclude *P. aeruginosa*, *Acinetobacter* spp., *Burkholderia cepacia* complex, and *Stenotrophomonas maltophilia* (refer to Tables 2B-2, 2B-3, and 2B-4, respectively). Recommendations for testing and reporting *Aeromonas* spp. (including members of *A. caviae* complex, *A. hydrophila* complex, and *A. veronii* complex), *Burkholderia mallei, Burkholderia pseudomallei,* and *Vibrio* spp. (including *V. cholerae*) are found in CLSI M45.[1]

(3) For other non-Enterobacterales, the disk diffusion method has not been systematically studied. Therefore, for this organism group, disk diffusion testing is not recommended.

NOTE: Information in boldface type is new or modified since the previous edition.

Table 2B-5. Non-Enterobacterales (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
		S	I	R	S	I	R	
PENICILLINS								
Piperacillin*	–	–	–	–	≤ 16	32–64	≥ 128	
β-LACTAM COMBINATION AGENTS								
(4) Organisms that test susceptible to the β-lactam agent alone are also considered susceptible to the β-lactam combination agent. However, organisms that test susceptible to the β-lactam combination agent cannot be assumed to be susceptible to the β-lactam agent alone. Similarly, organisms that test intermediate or resistant to the β-lactam agent alone may be susceptible to the β-lactam combination agent.								
Piperacillin-tazobactam	–	–	–	–	≤ 16/4	32/4–64/4	≥ 128/4	
Ticarcillin-clavulanate*	–	–	–	–	≤ 16/2	32/2–64/2	≥ 128/2	
CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.)								
Ceftazidime	–	–	–	–	≤ 8	16	≥ 32	
Cefepime	–	–	–	–	≤ 8	16	≥ 32	
Cefotaxime	–	–	–	–	≤ 8	16–32	≥ 64	
Ceftriaxone	–	–	–	–	≤ 8	16–32	≥ 64	
Cefoperazone*	–	–	–	–	≤ 16	32	≥ 64	
Ceftizoxime*	–	–	–	–	≤ 8	16–32	≥ 64	
Moxalactam*	–	–	–	–	≤ 8	16–32	≥ 64	
MONOBACTAMS								
Aztreonam	–	–	–	–	≤ 8	16	≥ 32	
CARBAPENEMS								
Imipenem	–	–	–	–	≤ 4	8	≥ 16	
Meropenem	–	–	–	–	≤ 4	8	≥ 16	
AMINOGLYCOSIDES								
Gentamicin	–	–	–	–	≤ 4	8	≥ 16	
Tobramycin	–	–	–	–	≤ 4	8	≥ 16	
Amikacin	–	–	–	–	≤ 16	32	≥ 64	
Netilmicin*	–	–	–	–	≤ 8	16	≥ 32	

Table 2B-5. Non-Enterobacterales (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
		S	I	R	S	I	R	
TETRACYCLINES								
(5) Isolates that **test** susceptible to tetracycline are considered susceptible to doxycycline and minocycline. **Isolates** that **test** intermediate or resistant to tetracycline **should be tested against doxycycline or minocycline if those results are needed for treatment.**								
Tetracycline (U)^{a}	–	–	–	–	≤ 4	8	≥ 16	
Doxycycline*	–	–	–	–	≤ 4	8	≥ 16	
Minocycline	–	–	–	–	≤ 4	8	≥ 16	
FLUOROQUINOLONES								
Ciprofloxacin Levofloxacin	– –	– –	–	–	≤ 1 ≤ 2	2 4	≥ 4 ≥ 8	
Gatifloxacin*	–	–	–	–	≤ 2	4	≥ 8	
Lomefloxacin*	–	–	–	–	≤ 2	4	≥ 8	
Norfloxacin* (U)^{a}	–	–	–	–	≤ 4	8	≥ 16	
Ofloxacin*	–	–	–	–	≤ 2	4	≥ 8	
FOLATE PATHWAY ANTAGONISTS								
Trimethoprim-sulfamethoxazole	–	–	–	–	≤ 2/38	–	≥ 4/76	
Sulfonamides (U)^{a}	–	–	–	–	≤ 256	–	≥ 512	
PHENICOLS								
Chloramphenicol*	–	–	–	–	≤ 8	16	≥ 32	**(6)** Not routinely reported on organisms isolated from the urinary tract.

Abbreviations: CAMHB, cation-adjusted Mueller-Hinton broth; I, intermediate; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; QC, quality control; R, resistant; S, susceptible; U, urine.

Symbol: *, designation for “Other” agents that are not included in Tables 1 but have established clinical breakpoints.

Table 2B-5. Non-Enterobacterales (Continued)

Footnote

a. Report only on organisms isolated from the urinary tract.

Reference for Table 2B-5

[1] CLSI. *Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria.* 3rd ed. CLSI guideline M45. Clinical and Laboratory Standards Institute; 2016.

Table 2C. Zone Diameter and MIC Breakpoints for *Staphylococcus* spp.

Testing Conditions

Medium: Disk diffusion: MHA
Broth dilution: CAMHB; CAMHB + 2% NaCl for oxacillin; CAMHB supplemented to 50 µg/mL calcium for daptomycin
Agar dilution: MHA; MHA + 2% NaCl for oxacillin
NOTE: Agar dilution has not been validated for daptomycin.

Inoculum: Colony suspension, equivalent to a 0.5 McFarland standard

Incubation: 35°C ± 2°C; ambient air
Disk diffusion: 16–18 hours; 24 hours (for cefoxitin when testing *Staphylococcus* spp., except *S. aureus,* ***S. coagulans,*** *S. lugdunensis, S. pseudintermedius,* and *S. schleiferi*)
Dilution methods: 16–20 hours; 24 hours for oxacillin and vancomycin
Testing at temperatures above 35°C may not detect MRS.

QC Recommendations

Refer to the following:

- **Tables 4A-1 and 5A-1 that list acceptable QC ranges applicable for each method**
- **Appendix I to develop a QC plan**

When a commercial test system is used for antimicrobial susceptibility testing, refer to the manufacturer's instructions for QC **strains** and QC ranges.

Refer to Tables 3G, 3H, 3I, 3J, and 3K for additional testing recommendations, reporting suggestions, and QC.

General Comments

(1) Refer to Table 1C for antimicrobial agents that should be considered for testing and reporting by microbiology laboratories.

(2) For disk diffusion, test a maximum of 12 disks on a 150-mm plate and no more than 6 disks on a 100-mm plate; disks should be placed no less than 24 mm apart, center to center (see CLSI M02[1]). Each zone diameter should be clearly measurable; overlapping zones prevent accurate measurement. Measure the diameter of the zones of complete inhibition (as judged by the unaided eye), including the diameter of the disk (see CLSI M02QG[2]). Hold the Petri plate a few inches above a black background illuminated with reflected light. The zone margin should be considered the area showing no obvious, visible growth that can be detected with the unaided eye. Ignore faint growth of tiny colonies that can be detected only with a magnifying lens at the edge of the zone of inhibited growth. With trimethoprim and the sulfonamides, antagonists in the medium may allow some slight growth; therefore, disregard slight growth (20% or less of the lawn of growth) and measure the more obvious margin to determine the zone diameter. For linezolid, any discernible growth within the zone of inhibition is indicative of resistance to the respective agent.

Table 2C. *Staphylococcus* spp. (Continued)

(3) *S. aureus* complex consists of the coagulase-positive species *S. aureus, S. argenteus,* and *S. schweitzeri* **and other species not listed.[3,4,5] At this time, CLSI has not evaluated the methods described herein on species other than *S. aureus*.** If *S. argenteus* is identified by MALDI-TOF MS or sequencing, it is recommended that it be reported as "*S. aureus* complex (*S. argenteus*)," and *S. aureus* phenotypic testing method recommendations, breakpoints, and interpretive categories should be used. Human infections with *S. schweitzeri* have yet to be reported.[6]

(4) For staphylococci when testing chloramphenicol, clindamycin, erythromycin, linezolid, tedizolid, and tetracycline by broth microdilution MIC, trailing growth can make end point determination difficult. In such cases, read the MIC at the lowest concentration where the trailing begins. Tiny buttons of growth should be ignored (see CLSI M07[7]). With trimethoprim and the sulfonamides, antagonists in the medium may allow some slight growth; therefore, read the end point at the concentration in which there is ≥ 80% reduction in growth compared with the control (see CLSI M07[7]).

(5) Routine testing of urine isolates of *S. saprophyticus* is not advised, because infections respond to concentrations achieved in urine of antimicrobial agents commonly used to treat acute, uncomplicated UTIs (eg, nitrofurantoin, trimethoprim-sulfamethoxazole, or a fluoroquinolone).

(6) Historically, **for *Staphylococcus aureus* and staphylococci other than *Staphylococcus aureus* (SOSA)** resistance to the penicillinase-stable penicillins (see Glossary I) has been referred to as "methicillin resistance" or "oxacillin resistance." MRS are strains that express *mecA* (or its homologue, *mecC*) or another mechanism of resistance, such as changes in affinity of penicillin-binding proteins for oxacillin (eg, modified *S. aureus* strains).

Most methicillin (oxacillin) resistance is mediated by *mecA*, encoding PBP2a (also called PBP2′). Tests for *mecA* and PBP2a are the most definitive tests for detection of methicillin (oxacillin) resistance for *Staphylococcus* spp. Mechanisms of methicillin (oxacillin) resistance other than *mecA*, such as *mecC*, are rare.[8] MICs for strains with *mecC* are typically cefoxitin resistant and oxacillin susceptible; *mecC* resistance cannot be detected by tests directed at *mecA* or PBP2a.

Isolates that test positive for *mecA*, *mecC*, or PBP2a or resistant by any of the recommended phenotypic methods should be reported as methicillin (oxacillin) resistant (see the table below and Appendix G).

MRS are resistant to currently available β-lactam agents, with the exception of ceftaroline (see comment 12). This is because most documented cases of MRS infections have responded poorly to β-lactam therapy or because convincing clinical data that document clinical efficacy for those agents have not been presented.

Detection of methicillin (oxacillin) resistance in staphylococci is achieved by using specific methods as listed in this table and further described in Table 3H.

Table 2C. *Staphylococcus* spp. (Continued)

	Methods or Targets for Detection of Methicillin (Oxacillin)-Resistant *Staphylococcus* spp.							
		Disk Diffusion		MIC				
	Organism	Cefoxitin	Oxacillin	Cefoxitin	Oxacillin	*mecA*	PBP2a	Oxacillin Salt Agar
	S. aureus	Yes (16–18 h)	No	Yes (16–20 h)	Yes (24 h)	Yes	Yes	Yes (24 h)
SOSA	*S. lugdunensis*	Yes (16–18 h)	No	Yes (16–20 h)	Yes (24 h)	Yes	Yes	No
	S. epidermidis	Yes (24 h)	Yes (16–18 h)	No	Yes (24 h)	Yes	Yes	No
	S. pseudintermedius	No	Yes (16–18 h)	No	Yes (24 h)	Yes	Yes	No
	S. coagulans *S. schleiferi*	No	Yes (16–18 h)	No	Yes (24 h)	Yes	Yes	No
	Staphylococcus spp. (not listed above or not identified to the species level)	Yes, with exceptions[a] (24 h)	No	No	Yes (24 h)	Yes	Yes	No

Abbreviations: h, hour(s); MIC, minimal inhibitory concentration; PBP2a, penicillin-binding protein 2a; **SOSA, staphylococci other than *Staphylococcus aureus***.

[a] The cefoxitin disk diffusion test may not perform reliably for all species (eg, *S. haemolyticus*) that fall into the category of "*Staphylococcus* spp. (not listed above or not identified to the species level)."[9]

(7) For tests for β-lactamase production, detection of methicillin (oxacillin) resistance using oxacillin salt agar, reduced susceptibility to vancomycin, ICR, and high-level mupirocin resistance, refer to Tables 3G, 3H, 3I, 3J, and 3K, respectively.

NOTE: Information in boldface type is new or modified since the previous edition.

Antimicrobial Agent	*Staphylococcus* spp. Indications	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm				Interpretive Categories and MIC Breakpoints, µg/mL				Comments
			S	SDD	I	R	S	SDD	I	R	
PENICILLINASE-LABILE PENICILLINS											
(8) Penicillin-susceptible staphylococci are susceptible to other β-lactam agents with established clinical efficacy for staphylococcal infections (including both penicillinase-labile and penicillinase-stable agents; see Glossary I). Penicillin-resistant staphylococci are resistant to penicillinase-labile penicillins. **(9)** Penicillin should be used to test the susceptibility of all staphylococci to penicillinase-labile penicillins (see Glossary I). Penicillin-resistant strains of staphylococci produce β-lactamase. Perform a test(s) to detect β-lactamase production on staphylococci for which the penicillin MICs are ≤ 0.12 µg/mL or zone diameters ≥ 29 mm before reporting the isolate as penicillin susceptible. Rare isolates of staphylococci that contain genes for β-lactamase production may test negative for β-lactamase. Consequently, for serious infections requiring penicillin therapy, perform MIC tests and β-lactamase tests on initial and all subsequent isolates from the same patient. PCR testing for the blaZ β-lactamase gene may be considered. See Table 3G.											
Penicillin	All staphylococci	10 units	≥ 29	–	–	≤ 28	≤ 0.12		–	≥ 0.25	**(10)** For MRS, report penicillin as resistant or do not report.

Table 2C. *Staphylococcus* spp. (Continued)

<table>
<tr><th rowspan="2">Antimicrobial Agent</th><th rowspan="2"><i>Staphylococcus</i> spp. Indications</th><th rowspan="2">Disk Content</th><th colspan="4">Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm</th><th colspan="4">Interpretive Categories and MIC Breakpoints, µg/mL</th><th rowspan="2">Comments</th></tr>
<tr><th>S</th><th>SDD</th><th>I</th><th>R</th><th>S</th><th>SDD</th><th>I</th><th>R</th></tr>
<tr><td colspan="12">PENICILLINASE-STABLE PENICILLINS</td></tr>
<tr><td colspan="12">(11) Cefoxitin is tested as a surrogate for oxacillin for some species of <i>Staphylococcus</i> (see table in general comment [6]). Isolates that test resistant by cefoxitin or oxacillin should be reported as methicillin (oxacillin) resistant. If testing only cefoxitin, report as methicillin (oxacillin) susceptible or resistant based on the cefoxitin result.
(12) Oxacillin (or cefoxitin) results can be applied to the other penicillinase-stable penicillins (cloxacillin, dicloxacillin, methicillin, and nafcillin). For agents with established clinical efficacy and considering site of infection and appropriate dosing, methicillin (oxacillin)-susceptible staphylococci can be considered susceptible to:
• β-Lactam combination agents (amoxicillin-clavulanate, ampicillin-sulbactam, piperacillin-tazobactam)
• Oral cephems (cefaclor, cefdinir, cephalexin, cefpodoxime, cefprozil, cefuroxime, loracarbef)
• Parenteral cephems including cephalosporins I, II, III, and IV (cefamandole, cefazolin, cefepime, cefmetazole, cefonicid, cefoperazone, cefotaxime, cefotetan, ceftizoxime, ceftriaxone, cefuroxime, ceftaroline, moxalactam)
• Carbapenems (doripenem, ertapenem, imipenem, meropenem)
MRS are resistant to currently available β-lactam antimicrobial agents, with the exception of ceftaroline. Thus, susceptibility or resistance to a wide array of β-lactam antimicrobial agents may be deduced from testing only penicillin and either cefoxitin or oxacillin. Testing of other β-lactam agents, except ceftaroline, is not advised. See general comment (6).
Additional explanation on the use of cefoxitin for prediction of <i>mecA</i>-mediated methicillin (oxacillin) resistance can be found in CLSI M02[1] and CLSI M07.[7]</td></tr>
<tr><td rowspan="2">Oxacillin</td><td rowspan="2"><i>S. aureus</i> and
<i>S. lugdunensis</i></td><td>–</td><td>–</td><td>–</td><td>–</td><td>–</td><td>≤ 2
(oxacillin)</td><td>–</td><td>–</td><td>≥ 4
(oxacillin)</td><td rowspan="2">(13) For isolates of <i>S. aureus</i> that do not grow well on CAMHB or unsupplemented MHA (eg, small-colony variants), testing on other media (eg, BMHA) does not reliably detect <i>mecA</i>-mediated resistance. Testing for PBP2a using induced growth (ie, growth taken from the zone margin surrounding a cefoxitin disk on either BMHA or a blood agar plate after 24 h incubation in 5% CO_2) or <i>mecA</i> should be done.
See general comment (6) and comments (8), (11), and (12).</td></tr>
<tr><td>30 µg cefoxitin (surrogate test for oxacillin)</td><td>≥ 22
(cefoxitin)</td><td>–</td><td>–</td><td>≤ 21
(cefoxitin)</td><td>≤ 4
(cefoxitin)</td><td>–</td><td>–</td><td>≥ 8
(cefoxitin)</td></tr>
</table>

Table 2C. *Staphylococcus* spp. (Continued)

Antimicrobial Agent	*Staphylococcus* spp. Indications	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm				Interpretive Categories and MIC Breakpoints, µg/mL				Comments
			S	SDD	I	R	S	SDD	I	R	
PENICILLINASE-STABLE PENICILLINS (Continued)											
Oxacillin	*S. epidermidis*	1 µg oxacillin	≥ 18 (oxacillin)	–	–	≤ 17 (oxacillin)	≤ 0.5 (oxacillin)	–	–	≥ 1 (oxacillin)	See general comment (6) and comments (8), (11), and (12).
		30 µg cefoxitin (surrogate test for oxacillin)	≥ 25 (cefoxitin)	–	–	≤ 24 (cefoxitin)	–	–	–	–	
	S. pseudintermedius, ***S. coagulans***, and *S. schleiferi*	1 µg oxacillin	≥ 18 (oxacillin)	–	–	≤ 17 (oxacillin)	≤ 0.5 (oxacillin)	–	–	≥ 1 (oxacillin)	See general comment (6) and comments (8), (11), and (12).
	Staphylococcus spp., except: *S. aureus* *S. lugdunensis* *S. epidermidis* *S. pseudintermedius* ***S. coagulans*** *S. schleiferi*	30 µg cefoxitin (surrogate test for oxacillin)	≥ 25 (cefoxitin)	–	–	≤ 24 (cefoxitin)	≤ 0.5 (oxacillin)	–	–	≥ 1 (oxacillin)	See general comment (6) and comments (8), (11), and (12).
CEPHEMS (PARENTERAL)											
Ceftaroline	*S. aureus*, including MRSA	30 µg	≥ 25	20–24	–	≤ 19	≤ 1	2–4	–	≥ 8	
GLYCOPEPTIDES											
(14) MIC tests should be performed to determine the susceptibility of all isolates of staphylococci to vancomycin. The disk test does not differentiate vancomycin-susceptible isolates of *S. aureus* from vancomycin-intermediate isolates, nor does the test differentiate among vancomycin-susceptible, -intermediate, and -resistant isolates of *Staphylococcus* spp. other than *S. aureus*, all of which give similar size zones of inhibition.											

Table 2C. *Staphylococcus* spp. (Continued)

Antimicrobial Agent	*Staphylococcus* spp. Indications	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm				Interpretive Categories and MIC Breakpoints, µg/mL				Comments
			S	SDD	I	R	S	SDD	I	R	
GLYCOPEPTIDES (Continued)											
Vancomycin	*S. aureus*, including MRSA	–	–	–	–	–	≤ 2	–	4–8	≥ 16	**(15)** For *S. aureus*, vancomycin-susceptible isolates may become vancomycin intermediate during the course of prolonged therapy. **(16)** Send any *S. aureus* for which the vancomycin is ≥ 8 µg/mL to a referral laboratory. See Appendix A. Also refer to Table 3I for *S. aureus*, CLSI M02,[1] and CLSI M07.[7]
	SOSA	–	–	–	–	–	≤ 4	–	8–16	≥ 32	**(17)** Send any **SOSA** for which the vancomycin MIC is ≥ 32 µg/mL to a referral laboratory. See Appendix A. See also CLSI M02[1] and CLSI M07.[7]
LIPOGLYCOPEPTIDES											
Dalbavancin	*S. aureus*, including MRSA	–	–	–	–	–	≤ 0.25	–	–	–	
Oritavancin		–	–	–	–	–	≤ 0.12	–	–	–	
Telavancin		–	–	–	–	–	≤ 0.12	–	–	–	
Teicoplanin (Inv.)	All staphylococci	–	–	–	–	–	≤ 8	–	16	≥ 32	
LIPOPEPTIDES											
Daptomycin	All staphylococci	–	–	–	–	–	≤ 1	–	–	–	**(18)** Not routinely reported on organisms isolated from the lower respiratory tract.
AMINOGLYCOSIDES											
(19) For staphylococci that test susceptible, gentamicin is used only in combination with other active agents that test susceptible.											
Gentamicin	All staphylococci	10 µg	≥ 15	–	13–14	≤ 12	≤ 4	–	8	≥ 16	

Table 2C. *Staphylococcus* spp. (Continued)

Antimicrobial Agent	*Staphylococcus* spp. Indications	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm				Interpretive Categories and MIC Breakpoints, μg/mL				Comments
			S	SDD	I	R	S	SDD	I	R	
MACROLIDES											
(20) Not routinely reported on organisms isolated from the urinary tract.											
Azithromycin or	All staphylococci	15 μg	≥ 18	–	14–17	≤ 13	≤ 2	–	4	≥ 8	
clarithromycin		15 μg	≥ 18		14–17	≤ 13	≤ 2		4	≥ 8	
or erythromycin		15 μg	≥ 23		14–22	≤ 13	≤ 0.5		1–4	≥ 8	
Dirithromycin*		15 μg	≥ 19	–	16–18	≤ 15	≤ 2	–	4	≥ 8	
TETRACYCLINES											
(21) Isolates that **test** susceptible to tetracycline are considered susceptible to doxycycline and minocycline. **Isolates** that **test** intermediate or resistant to tetracycline **should be tested against doxycycline or minocycline if those results are needed for treatment.**											
Tetracycline	All staphylococci	30 μg	≥ 19	–	15–18	≤ 14	≤ 4	–	8	≥ 16	
Doxycycline		30 μg	≥ 16	–	13–15	≤ 12	≤ 4	–	8	≥ 16	
Minocycline		30 μg	≥ 19	–	15–18	≤ 14	≤ 4	–	8	≥ 16	See comment (20).
FLUOROQUINOLONES											
(22) *Staphylococcus* spp. may develop resistance during therapy with quinolones. Therefore, isolates that are initially susceptible may become resistant within a few days after initiation of therapy. Testing of repeat isolates may be warranted.											
Ciprofloxacin or	All staphylococci	5 μg	≥ 21	–	16–20	≤ 15	≤ 1	–	2	≥ 4	
levofloxacin		5 μg	≥ 19	–	16–18	≤ 15	≤ 1	–	2	≥ 4	
Moxifloxacin		5 μg	≥ 24	–	21–23	≤ 20	≤ 0.5	–	1	≥ 2	
Enoxacin* (U)[a]		10 μg	≥ 18	–	15–17	≤ 14	≤ 2	–	4	≥ 8	
Gatifloxacin*		5 μg	≥ 23	–	20–22	≤ 19	≤ 0.5	–	1	≥ 2	
Grepafloxacin*		5 μg	≥ 18	–	15–17	≤ 14	≤ 1	–	2	≥ 4	
Lomefloxacin*		10 μg	≥ 22	–	19–21	≤ 18	≤ 2	–	4	≥ 8	
Norfloxacin* (U)[a]		10 μg	≥ 17	–	13–16	≤ 12	≤ 4	–	8	≥ 16	
Ofloxacin*		5 μg	≥ 18	–	15–17	≤ 14	≤ 1	–	2	≥ 4	
Sparfloxacin*		5 μg	≥ 19	–	16–18	≤ 15	≤ 0.5	–	1	≥ 2	
Fleroxacin (Inv.)		5 μg	≥ 19	–	16–18	≤ 15	≤ 2	–	4	≥ 8	

Table 2C. *Staphylococcus* spp. (Continued)

Antimicrobial Agent	*Staphylococcus* spp. Indications	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm				Interpretive Categories and MIC Breakpoints, µg/mL				Comments
			S	SDD	I	R	S	SDD	I	R	
NITROFURANS											
Nitrofurantoin (U)[a]	All staphylococci	300 µg	≥ 17	–	15–16	≤ 14	≤ 32	–	64	≥ 128	
LINCOSAMIDES											
Clindamycin	All staphylococci	2 µg	≥ 21	–	15–20	≤ 14	≤ 0.5	–	1–2	≥ 4	**(23)** For isolates that test erythromycin resistant and clindamycin susceptible or intermediate, testing for ICR by disk diffusion using the D-zone test or by broth microdilution is required before reporting clindamycin (see Table 3J, CLSI M02,[1] and CLSI M07[7]). See comment (20).
FOLATE PATHWAY ANTAGONISTS											
Trimethoprim-sulfamethoxazole	All staphylococci	1.25/23.75 µg	≥ 16	–	11–15	≤ 10	≤ 2/38	–	–	≥ 4/76	
Sulfonamides (U)[a]		250 or 300 µg	≥ 17	–	13–16	≤ 12	≤ 256	–	–	≥ 512	
Trimethoprim (U)[a]		5 µg	≥ 16	–	11–15	≤ 10	≤ 8	–	–	≥ 16	
PHENICOLS											
Chloramphenicol*	All staphylococci	30 µg	≥ 18	–	13–17	≤ 12	≤ 8	–	16	≥ 32	See comment (20).
ANSAMYCINS											
Rifampin	All staphylococci	5 µg	≥ 20	–	17–19	≤ 16	≤ 1	–	2	≥ 4	**(24)** ***Rx:*** Rifampin should not be used alone for antimicrobial therapy.

Table 2C. *Staphylococcus* spp. (Continued)

Antimicrobial Agent	*Staphylococcus* spp. Indications	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm				Interpretive Categories and MIC Breakpoints, µg/mL				Comments
			S	SDD	I	R	S	SDD	I	R	
STREPTOGRAMINS											
Quinupristin-dalfopristin*	*S. aureus*	15 µg	≥ 19	–	16–18	≤ 15	≤ 1	–	2	≥ 4	**(25)** Report only on MSSA.
OXAZOLIDINONES											
(26) *S. aureus* that test susceptible to linezolid are considered susceptible to tedizolid. **Isolates** that test resistant to linezolid **should be tested against tedizolid if that result is needed for treatment.**											
Linezolid	All staphylococci	30 µg	≥ 26	–	23–25	≤ 22	≤ 4	–	–	≥ 8	
Tedizolid	*S. aureus*, including MRSA	2 µg	≥ 19	–	16–18	≤ 15	≤ 0.5	–	1	≥ 2	
PLEUROMUTILINS											
Lefamulin	*S. aureus*, including MRSA	20 µg	≥ 23	–	–	–	≤ 0.25	–	–	–	See comment (20).

Abbreviations: BMHA, blood Mueller-Hinton agar; CAMHB, cation-adjusted Mueller-Hinton broth; CO_2, carbon dioxide; h, hour(s); I, intermediate; ICR, inducible clindamycin resistance; Inv., investigational agent; MALDI-TOF MS, matrix-assisted laser-desorption/ionization time-of-flight mass spectrometry; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; MRS, methicillin (oxacillin)-resistant staphylococci; MRSA, methicillin (oxacillin)-resistant *Staphylococcus aureus*; MSSA, methicillin (oxacillin)-susceptible *Staphylococcus aureus*; NaCl, sodium chloride; PBP2a, penicillin-binding protein 2a; PCR, polymerase chain reaction; QC, quality control; R, resistant; S, susceptible; SDD, susceptible-dose dependent; **SOSA, staphylococci other than *Staphylococcus aureus***; U, urine; UTI, urinary tract infection.
Symbol: *, designation for "Other" agents that are not included in Tables 1 but have established clinical breakpoints.

Footnote

a. Report only on organisms isolated from the urinary tract.

References for Table 2C

[1] CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests*. 14th ed. CLSI standard M02. Clinical and Laboratory Standards Institute; 2024.

[2] CLSI. *M02 Disk Diffusion Reading Guide*. 2nd ed. CLSI quick guide M02-Ed14-QG. Clinical and Laboratory Standards Institute; 2024.

[3] **Schutte AHJ, Strepis N, Zandijk WHA, Bexkens ML, Bode LGM, Klaassen CHW. Characterization of *Staphylococcus roterodami* sp. nov., a new species within the *Staphylococcus aureus* complex isolated from a human foot infection. *Int J Syst Evol Microbiol*. 2021;71(9). doi:10.1099/ijsem.0.004996**

Table 2C. *Staphylococcus* spp. (Continued)

[4] **Chew KL, Octavia S, Lai D, Lin RTP, Teo JWP. *Staphylococcus singaporensis* sp. nov., a new member of the *Staphylococcus aureus* complex, isolated from human clinical specimens. *Int J Syst Evol Microbiol*. 2021;71(10). doi:10.1099/ijsem.0.005067**

[5] **Akoua-Koffi C, Kacou N'Douba A, Djaman JA, Herrmann M, Schaumburg F, Niemann S. *Staphylococcus schweitzeri*—an emerging one health pathogen? *Microorganisms*. 2022;10(4):770. doi:10.3390/microorganisms10040770**

[6] Becker K, Schaumburg F, Kearns A, et al. Implications of identifying the recently defined members of the *Staphylococcus aureus* complex *S. argenteus* and *S. schweitzeri*: a position paper of members of the ESCMID Study Group for Staphylococci and Staphylococcal Diseases (ESGS). *Clin Microbiol Infect*. 2019;25(9):1064-1070. doi:10.1016/j.cmi.2019.02.028

[7] CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*. 12th ed. CLSI standard M07. Clinical and Laboratory Standards Institute; 2024.

[8] García-Álvarez L, Holden MTG, Lindsay H, et al. Meticillin-resistant *Staphylococcus aureus* with a novel *mecA* homologue in human and bovine populations in the UK and Denmark: a descriptive study. *Lancet Infect Dis*. 2011;11(8):595-603. doi:10.1016/S1473-3099(11)70126-8

[9] Humphries RM, Magnano P, Burnham CA, et al. Evaluation of surrogate tests for the presence of *mecA*-mediated methicillin resistance in *Staphylococcus capitis, Staphylococcus haemolyticus, Staphylococcus hominis,* and *Staphylococcus warneri*. *J Clin Microbiol*. 2020;59(1):e02290-20. doi:10.1128/JCM.02290-20

Table 2D. Zone Diameter and MIC Breakpoints for *Enterococcus* spp.

Testing Conditions

Medium: Disk diffusion: MHA
Broth dilution: CAMHB; CAMHB supplemented to 50 µg/mL calcium for daptomycin
Agar dilution: MHA; agar dilution has not been validated for daptomycin

Inoculum: Broth culture method or colony suspension, equivalent to a 0.5 McFarland standard

Incubation: 35°C ± 2°C; ambient air
Disk diffusion: 16–18 hours
Dilution methods: 16–20 hours
All methods: 24 hours for vancomycin

QC Recommendations

Refer to the following:

- **Tables 4A-1 and 5A-1 that list acceptable QC ranges applicable for each method**
- **Appendix I to develop a QC plan**

When a commercial test system is used for antimicrobial susceptibility testing, refer to the manufacturer's instructions for QC **strains** and QC ranges.

Refer to Tables 3I and 3L for additional testing recommendations, reporting suggestions, and QC.

General Comments

(1) Refer to Table 1D for antimicrobial agents that should be considered for testing and reporting by microbiology laboratories.

(2) For disk diffusion, test a maximum of 12 disks on a 150-mm plate and no more than 6 disks on a 100-mm plate; disks should be placed no less than 24 mm apart, center to center (see CLSI M02[1]). Each zone diameter should be clearly measurable; overlapping zones prevent accurate measurement. Measure the diameter of the zones of complete inhibition (as judged by the unaided eye), including the diameter of the disk (see CLSI M02QG[2]). Hold the Petri plate a few inches above a black background illuminated with reflected light, except for vancomycin, which should be read with transmitted light (plate held up to light source). The zone margin should be considered the area showing no obvious, visible growth that can be detected with the unaided eye. Ignore faint growth of tiny colonies that can be detected only with a magnifying lens at the edge of the zone of inhibited growth. Any discernible growth within the zone of inhibition indicates vancomycin resistance.

(3) For enterococci when testing chloramphenicol, erythromycin, linezolid, tedizolid, and tetracycline by broth microdilution MIC, trailing growth can make end-point determination difficult. In such cases, read the MIC at the lowest concentration where the trailing begins. Tiny buttons of growth should be ignored (see CLSI M07[3]).

Table 2D. *Enterococcus* spp. (Continued)

(4) **WARNING:** For *Enterococcus* spp., aminoglycosides (except for high-level resistance testing), cephalosporins, clindamycin, and trimethoprim-sulfamethoxazole may appear active *in vitro*, but they are not effective clinically, and isolates should not be reported as susceptible.

(5) Synergy between **a cell wall–active agent (eg,** ampicillin, penicillin, or vancomycin) and an aminoglycoside can be predicted for enterococci by using a high-level aminoglycoside (gentamicin and streptomycin) test (see Table 3L).

(6) An intermediate (I) with a ^ in Tables 2 indicates agents that have the potential to concentrate in the urine. The I^ is for informational use only. The decision to report I^ is best made by each laboratory based on institution-specific guidelines and in consultation with appropriate medical personnel.

NOTE: Information in boldface type is new or modified since the previous edition.

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL				Comments
		S	I	R	S	SDD	I	R	
PENICILLINS									
Penicillin	10 units	≥ 15	–	≤ 14	≤ 8	–	–	≥ 16	**(7)** The results of ampicillin susceptibility tests should be used to predict the activity of amoxicillin. Ampicillin results may be used to predict susceptibility to amoxicillin-clavulanate, ampicillin-sulbactam, and piperacillin-tazobactam among non–β-lactamase-producing enterococci. Ampicillin susceptibility can be used to predict imipenem susceptibility, providing the species is confirmed to be *E. faecalis*. **(8)** Enterococci susceptible to penicillin are predictably susceptible to ampicillin, amoxicillin, ampicillin-sulbactam, amoxicillin-clavulanate, and piperacillin-tazobactam for non–β-lactamase-producing enterococci. However, enterococci susceptible to ampicillin cannot be assumed to be susceptible to penicillin. If penicillin results are needed, testing of penicillin is required. **(9)** ***Rx:*** Combination therapy with high-dosage parenteral ampicillin, amoxicillin, penicillin, or vancomycin, plus an aminoglycoside, may be indicated for serious enterococcal infections, such as endocarditis, unless high-level resistance to both gentamicin and streptomycin is documented; such combinations are predicted to result in synergistic killing of enterococci. Refer to Table 3L for HLAR testing.
Ampicillin	10 µg	≥ 17	–	≤ 16	≤ 8	–	–	≥ 16	

Table 2D. *Enterococcus* spp. (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL				Comments
		S	I	R	S	SDD	I	R	
PENICILLINS (Continued)									
Penicillin	10 units	≥ 15	–	≤ 14	≤ 8	–	–	≥ 16	**(10)** Penicillin or ampicillin resistance among enterococci due to β-lactamase production has been reported very rarely. Penicillin or ampicillin resistance due to β-lactamase production is not reliably detected with routine disk or dilution methods but is detected using a direct, nitrocefin-based β-lactamase test. Because of the rarity of β-lactamase–positive enterococci, this test does not need to be performed routinely but can be used in selected cases. A positive β-lactamase test predicts resistance to penicillin as well as amino- and ureidopenicillins (see Glossary I).
Ampicillin	10 µg	≥ 17	–	≤ 16	≤ 8	–	–	≥ 16	
GLYCOPEPTIDES									
Vancomycin	30 µg	≥ 17	15–16	≤ 14	≤ 4	–	8–16	≥ 32	**(11)** When testing vancomycin against enterococci, plates should be held a full 24 h for accurate detection of resistance. Zones should be examined using transmitted light; the presence of a haze or any growth within the zone of inhibition indicates resistance. Organisms with intermediate zones should be tested by an MIC method as described in CLSI M07.[3] For isolates for which the vancomycin MICs are 8–16 µg/mL, perform biochemical tests for identification as listed under the "Vancomycin MIC ≥ 8 µg/mL" test found in Table 3I. See general comment (5) and comment (9).
LIPOGLYCOPEPTIDES									
Dalbavancin	–	–	–	–	≤ 0.25	–	–	–	**(12)** Report only on vancomycin-susceptible *E. faecalis*.
Oritavancin	–	–	–	–	≤ 0.12	–	–	–	See comment (12).
Telavancin	–	–	–	–	≤ 0.25	–	–	–	See comment (12).
Teicoplanin (Inv.)	30 µg	≥ 14	11–13	≤ 10	≤ 8	–	16	≥ 32	

Table 2D. *Enterococcus* spp. (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL				Comments
		S	I	R	S	SDD	I	R	
LIPOPEPTIDES									
Daptomycin *E. faecium* only	–	–	–	–	–	≤ 4	–	≥ 8	**(13)** Not routinely reported on organisms isolated from the lower respiratory tract. **(14)** The breakpoint for SDD is intended for serious infections due to *E. faecium*. Consultation with an infectious diseases specialist is recommended.
Daptomycin *Enterococcus* spp. other than *E. faecium*	–	–	–	–	≤ 2	–	4	≥ 8	See comment (13).
MACROLIDES									
Erythromycin*	15 µg	≥ 23	14–22	≤ 13	≤ 0.5	–	1–4	≥ 8	**(15)** Not routinely reported on organisms isolated from the urinary tract.
TETRACYCLINES									
(16) Isolates that **test** susceptible to tetracycline are considered susceptible to doxycycline and minocycline. **Isolates** that **test** intermediate or resistant to tetracycline **should be tested against doxycycline or minocycline if those results are needed for treatment.**									
Tetracycline (U)[a]	30 µg	≥ 19	15–18	≤ 14	≤ 4	–	8	≥ 16	
Doxycycline*	30 µg	≥ 16	13–15	≤ 12	≤ 4	–	8	≥ 16	
Minocycline*	30 µg	≥ 19	15–18	≤ 14	≤ 4	–	8	≥ 16	
FLUOROQUINOLONES									
Ciprofloxacin (U)[a] Levofloxacin (U)[a]	5 µg 5 µg	≥ 21 ≥ 17	16–20^ 14–16^	≤ 15 ≤ 13	≤ 1 ≤ 2	–	2^ 4^	≥ 4 ≥ 8	
Gatifloxacin*	5 µg	≥ 18	15–17^	≤ 14	≤ 2	–	4^	≥ 8	
Norfloxacin* (U)[a]	10 µg	≥ 17	13–16	≤ 12	≤ 4	–	8	≥ 16	
NITROFURANS									
Nitrofurantoin (U)[a]	300 µg	≥ 17	15–16	≤ 14	≤ 32	–	64	≥ 128	

Table 2D. *Enterococcus* spp. (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL				Comments
		S	I	R	S	SDD	I	R	
ANSAMYCINS									
Rifampin*	5 µg	≥ 20	17–19	≤ 16	≤ 1	–	2	≥ 4	**(17)** ***Rx:*** Rifampin should not be used alone for antimicrobial therapy.
FOSFOMYCINS									
Fosfomycin (U)[a]	200 µg	≥ 16	13–15	≤ 12	≤ 64	–	128	≥ 256	**(18)** Report only on *E. faecalis*. **(19)** The approved MIC testing method is agar dilution. Agar media should be supplemented with 25 µg/mL of glucose-6-phosphate. Broth dilution testing should not be performed. **(20)** The 200-µg fosfomycin disk contains 50 µg glucose-6-phosphate.
PHENICOLS									
Chloramphenicol*	30 µg	≥ 18	13–17	≤ 12	≤ 8	–	16	≥ 32	See comment (15).
STREPTOGRAMINS									
Quinupristin-dalfopristin*	15 µg	≥ 19	16–18	≤ 15	≤ 1	–	2	≥ 4	**(21)** Report only on vancomycin-resistant *E. faecium*.
OXAZOLIDINONES									
(22) *E. faecalis* that test susceptible to linezolid are considered susceptible to tedizolid. **Isolates** that **test** intermediate or resistant to linezolid **should be tested against tedizolid if that result is needed for treatment.**									
Linezolid	30 µg	≥ 23	21–22	≤ 20	≤ 2	–	4	≥ 8	
Tedizolid	–	–	–	–	≤ 0.5	–	–	–	See comment (18).

Abbreviations: CAMHB, cation-adjusted Mueller-Hinton broth; h, hour(s); HLAR, high-level aminoglycoside resistance; I, intermediate; Inv., investigational agent; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; QC, quality control; R, resistant; S, susceptible; SDD, susceptible-dose dependent; U, urine.

Symbols: ^, designation for agents that have the potential to concentrate in the urine; *, designation for "Other" agents not included in Tables 1 but have established clinical breakpoints.

Table 2D. *Enterococcus* spp. (Continued)

Footnote

a. Report only on organisms isolated from the urinary tract.

References for Table 2D

[1] CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests*. 14th ed. CLSI standard M02. Clinical and Laboratory Standards Institute; 2024.

[2] CLSI. *M02 Disk Diffusion Reading Guide*. 2nd ed. CLSI quick guide M02QG. Clinical and Laboratory Standards Institute; 2024.

[3] CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*. 12th ed. CLSI standard M07. Clinical and Laboratory Standards Institute; 2024.

Table 2E. Zone Diameter and MIC Breakpoints for *Haemophilus influenzae* and *Haemophilus parainfluenzae*

Testing Conditions

Medium:	Disk diffusion: HTM (for all agents when testing *H. influenzae* or *H. parainfluenzae*) or MH-F agar (MHA with 5% mechanically defibrinated horse blood and 20 µg/mL NAD) (for selected agents when testing *H. influenzae*) Broth dilution: HTM broth (for all agents when testing *H. influenzae* or *H. parainfluenzae*) or MH-F broth (for selected agents when testing *H. influenzae*)
Inoculum:	Colony suspension, equivalent to a 0.5 McFarland standard prepared using colonies from an overnight (preferably 20- to 24-hour) chocolate agar plate (see general comment [3])
Incubation:	35°C ± 2°C Disk diffusion: 5% CO_2; 16–18 hours Broth dilution: ambient air; 20–24 hours

QC Recommendations

Refer to the following:

- **Tables 4B and 5B that list acceptable QC ranges applicable for each method**
- **Appendix I to develop a QC plan**

When a commercial test system is used for antimicrobial susceptibility testing, refer to the manufacturer's instructions for QC **strains** and QC ranges.

General Comments

(1) Refer to Table 1E for antimicrobial agents that should be considered for testing and reporting by microbiology laboratories.

(2) *Haemophilus* spp., as used in this table, includes only *H. influenzae* and *H. parainfluenzae*. See CLSI M45[1] for testing and reporting recommendations for other species of *Haemophilus*.

(3) The 0.5 McFarland suspension contains approximately 1 to 4 × 10^8 CFU/mL. Use care in preparing this suspension, because higher inoculum concentrations may lead to false-resistant results with some β-lactam antimicrobial agents, particularly when β-lactamase–producing strains of *H. influenzae* are tested.

Table 2E. *Haemophilus influenzae* and *Haemophilus parainfluenzae* (Continued)

(4) For disk diffusion, test a maximum of 9 disks on a 150-mm plate and 4 disks on a 100-mm plate. Measure the diameter of the zones of complete inhibition (as judged by the unaided eye), including the diameter of the disk. Hold the Petri plate a few inches above a black background illuminated with reflected light. The zone margin should be considered the area showing no obvious, visible growth that can be detected with the unaided eye. Ignore faint growth of tiny colonies that can be detected only with a magnifying lens at the edge of the zone of inhibited growth. With trimethoprim and the sulfonamides, antagonists in the medium may allow some slight growth; therefore, disregard slight growth (20% or less of the lawn of growth) and measure the more obvious margin to determine the zone diameter.

(5) For isolates of *H. influenzae* from CSF, only results of testing with ampicillin, any of the third-generation cephalosporins listed below, chloramphenicol, and meropenem are appropriate to report.

(6) Amoxicillin-clavulanate, azithromycin, cefaclor, cefdinir, cefixime, cefpodoxime, cefprozil, cefuroxime, and clarithromycin are used as empiric therapy for respiratory tract infections due to *Haemophilus* spp. The results of susceptibility tests with these antimicrobial agents are often not necessary for management of individual patients.

(7) To make HTM: Prepare a fresh hematin stock solution by dissolving 50 mg of hematin powder in 100 mL of 0.01 mol/L NaOH with heat and stirring until the powder is thoroughly dissolved. Add 30 mL of the hematin stock solution and 5 g of yeast extract to 1 L of MHA, and autoclave. After autoclaving and cooling, add 3 mL of an NAD stock solution (50 mg NAD dissolved in 10 mL distilled water, filter sterilized) aseptically.

(8) For MIC testing with *H. influenzae*, results for ampicillin, amoxicillin-clavulanate, cefotaxime, ceftriaxone, cefuroxime, clarithromycin, chloramphenicol, levofloxacin, meropenem, rifampin, tetracycline, and trimethoprim-sulfamethoxazole were equivalent when HTM or MH-F broth and testing conditions and MIC breakpoints in this table were used. MICs obtained for cefuroxime and rifampin using MH-F broth may show a one-doubling dilution bias toward more resistance compared with HTM broth. The comparative study showed ≥ 90% essential agreement of MICs between MH-F broth and HTM broth for all agents listed above. MIC QC ranges for *H. influenzae* ATCC®[a] 49247 in Table 5B apply to testing using either HTM or MH-F broth.

(9) For disk diffusion testing with *H. influenzae*, results for ampicillin, ceftriaxone, cefuroxime, clarithromycin, chloramphenicol, levofloxacin, and tetracycline were equivalent when HTM or MH-F agar and the disk contents, testing conditions, and zone diameter breakpoints in this table were used. Results with trimethoprim-sulfamethoxazole were not equivalent between media, and HTM agar should be used if this agent is tested. Disk diffusion QC ranges for *H. influenzae* ATCC® 49247 in Table 4B apply to testing using either HTM or MH-F agar, with the exception of trimethoprim-sulfamethoxazole, which must be tested on HTM agar, not MH-F agar.

NOTE: Information in boldface type is new or modified since the previous edition.

Table 2E. *Haemophilus influenzae* and *Haemophilus parainfluenzae* (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
		S	I	R	S	I	R	
PENICILLINS								
Ampicillin	10 µg	≥ 22	19–21	≤ 18	≤ 1	2	≥ 4	See general comment (5). **(10)** The results of ampicillin susceptibility tests should be used to predict the activity of amoxicillin. The majority of isolates of *H. influenzae* that are resistant to ampicillin and amoxicillin produce a TEM-type β-lactamase. In most cases, a β-lactamase test can provide a rapid means of detecting resistance to ampicillin and amoxicillin. **(11)** Rare BLNAR strains of *H. influenzae* should be considered resistant to amoxicillin-clavulanate, ampicillin-sulbactam, cefaclor, cefamandole, cefetamet, cefonicid, cefprozil, cefuroxime, loracarbef, and piperacillin-tazobactam, despite apparent *in vitro* susceptibility of some BLNAR strains to these agents.
β-LACTAM COMBINATION AGENTS								
(12) Organisms that test susceptible to the β-lactam agent alone are also considered susceptible to the β-lactam combination agent. However, organisms that test susceptible to the β-lactam combination agent cannot be assumed to be susceptible to the β-lactam agent alone. Similarly, organisms that test intermediate or resistant to the β-lactam agent alone may be susceptible to the β-lactam combination agent.								
Ampicillin-sulbactam	10/10 µg	≥ 20	–	≤ 19	≤ 2/1	–	≥ 4/2	See comment (11).
Amoxicillin-clavulanate	20/10 µg	–	–	–	≤ 2/1	4/2	≥ 8/4	**(13)** Additional disk correlate data are pending before disk diffusion breakpoints with the dosage regimen listed in Table 2 Dosages can be established. See general comment (6) and comment (11).
Ceftolozane-tazobactam	–	–	–	–	≤ 0.5/4	–	–	**(14)** Report only on *H. influenzae*.
Piperacillin-tazobactam*	100/10 µg	≥ 21	–	–	≤ 1/4	–	≥ 2/4	See comment (11).

Table 2E. *Haemophilus influenzae* and *Haemophilus parainfluenzae* (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
		S	I	R	S	I	R	
CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.)								
Cefotaxime or ceftazidime or ceftriaxone	30 µg 30 µg 30 µg	≥ 26 ≥ 26 ≥ 26	– – –	– – –	≤ 2 ≤ 2 ≤ 2	– – –	– – –	See general comment (5).
Cefuroxime	30 µg	≥ 20	17–19	≤ 16	≤ 4	8	≥ 16	See general comments (6) and (8) and comment (11).
Ceftaroline	30 µg	≥ 30	–	–	≤ 0.5	–	–	See comment (14).
Cefonicid*	30 µg	≥ 20	17–19	≤ 16	≤ 4	8	≥ 16	See comment (11).
Cefamandole*	–	–	–	–	≤ 4	8	≥ 16	See comment (11).
Cefepime*	30 µg	≥ 26	–	–	≤ 2	–	–	
Ceftizoxime*	30 µg	≥ 26	–	–	≤ 2	–	–	See general comment (5).
CEPHEMS (ORAL)								
Cefaclor Cefprozil	30 µg 30 µg	≥ 20 ≥ 18	17–19 15–17	≤ 16 ≤ 14	≤ 8 ≤ 8	16 16	≥ 32 ≥ 32	See general comment (6) and comment (11).
Cefdinir or cefixime or cefpodoxime	5 µg 5 µg 10 µg	≥ 20 ≥ 21 ≥ 21	– – –	– – –	≤ 1 ≤ 1 ≤ 2	– – –	– – –	See general comment (6).
Cefuroxime	30 µg	≥ 20	17–19	≤ 16	≤ 4	8	≥ 16	See general comment (6) and comment (11).
Loracarbef*	30 µg	≥ 19	16–18	≤ 15	≤ 8	16	≥ 32	See general comment (6) and comment (11).
Ceftibuten*	30 µg	≥ 28	–	–	≤ 2	–	–	
Cefetamet (Inv.)	10 µg	≥ 18	15–17	≤ 14	≤ 4	8	≥ 16	See comment (11).
MONOBACTAMS								
Aztreonam	30 µg	≥ 26	–	–	≤ 2	–	–	
CARBAPENEMS								
Meropenem	10 µg	≥ 20	–	–	≤ 0.5	–	–	See general comment (5).
Ertapenem or imipenem	10 µg 10 µg	≥ 19 ≥ 16	– –	– –	≤ 0.5 ≤ 4	– –	– –	
Doripenem*	10 µg	≥ 16	–	–	≤ 1	–	–	

Table 2E. *Haemophilus influenzae* and *Haemophilus parainfluenzae* (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
		S	I	R	S	I	R	
MACROLIDES								
Azithromycin	15 µg	≥ 12	–	–	≤ 4	–	–	See general comment (6).
Clarithromycin	15 µg	≥ 13	11–12	≤ 10	≤ 8	16	≥ 32	
TETRACYCLINES								
(15) Isolates that **test** susceptible to tetracycline are considered susceptible to doxycycline and minocycline.								
Tetracycline	30 µg	≥ 29	26–28	≤ 25	≤ 2	4	≥ 8	
FLUOROQUINOLONES								
Ciprofloxacin or	5 µg	≥ 21	–	–	≤ 1	–	–	
levofloxacin or	5 µg	≥ 17	–	–	≤ 2	–	–	
moxifloxacin	5 µg	≥ 18	–	–	≤ 1	–	–	
Gemifloxacin*	5 µg	≥ 18	–	–	≤ 0.12	–	–	
Gatifloxacin*	5 µg	≥ 18	–	–	≤ 1	–	–	
Grepafloxacin*	5 µg	≥ 24	–	–	≤ 0.5	–	–	
Lomefloxacin*	10 µg	≥ 22	–	–	≤ 2	–	–	
Ofloxacin*	5 µg	≥ 16	–	–	≤ 2	–	–	
Sparfloxacin*	–	–	–	–	≤ 0.25	–	–	
Trovafloxacin*	10 µg	≥ 22	–	–	≤ 1	–	–	
Fleroxacin (Inv.)	5 µg	≥ 19	–	–	≤ 2	–	–	
FOLATE PATHWAY ANTAGONISTS								
Trimethoprim-sulfamethoxazole	1.25/ 23.75 µg	≥ 16	11–15	≤ 10	≤ 0.5/9.5	1/19–2/38	≥ 4/76	See general comment (9).
PHENICOLS								
Chloramphenicol*	30 µg	≥ 29	26–28	≤ 25	≤ 2	4	≥ 8	See general comment (5). **(16)** Not routinely reported on organisms isolated from the urinary tract.

Table 2E. *Haemophilus influenzae* and *Haemophilus parainfluenzae* (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
		S	I	R	S	I	R	
ANSAMYCINS								
Rifampin	5 µg	≥ 20	17–19	≤ 16	≤ 1	2	≥ 4	See general comment (8). **(17)** May be appropriate only for prophylaxis of case contacts. These breakpoints do not apply to therapy of patients with invasive *H. influenzae* disease.
PLEUROMUTILINS								
Lefamulin	20 µg	≥ 18	–	–	≤ 2	–	–	See comments (14) and (16).

Abbreviations: ATCC®, American Type Culture Collection; BLNAR, β-lactamase negative, ampicillin-resistant; CFU, colony-forming unit(s); CO_2, carbon dioxide; CSF, cerebrospinal fluid; HTM, *Haemophilus* test medium; I, intermediate; Inv., investigational agent; MHA, Mueller-Hinton agar; MH-F, Mueller-Hinton fastidious; MIC, minimal inhibitory concentration; NAD, β-nicotinamide adenine dinucleotide; NaOH, sodium hydroxide; QC, quality control; R, resistant; S, susceptible.
Symbol: *, designation for "Other" agents that are not included in Tables 1 but have established clinical breakpoints.

Footnote

a. ATCC® is a registered trademark of the American Type Culture Collection.

Reference for Table 2E

[1] CLSI. *Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria.* 3rd ed. CLSI guideline M45. Clinical and Laboratory Standards Institute; 2016.

Table 2F. Zone Diameter and MIC Breakpoints for *Neisseria gonorrhoeae*

Testing Conditions

Medium: Disk diffusion: GC agar base and 1% defined growth supplement (The use of a cysteine-free growth supplement is not required for disk diffusion testing.)
Agar dilution: GC agar base and 1% defined growth supplement (The use of a cysteine-free growth supplement is required for agar dilution tests with carbapenems and clavulanate. Cysteine-containing defined growth supplement does not significantly alter dilution test results with other drugs.)

Inoculum: Colony suspension, equivalent to a 0.5 McFarland standard prepared in MHB or 0.9% phosphate-buffered saline, pH 7, using colonies from an overnight (20- to 24-hour) chocolate agar plate incubated in 5% CO_2

Incubation: 36°C ± 1°C (do not exceed 37°C); 5% CO_2; all methods, 20–24 hours

QC Recommendations

Refer to the following:

- **Tables 4B and 5C that list acceptable QC ranges applicable for each method**
- **Appendix I to develop a QC plan**

When a commercial test system is used for antimicrobial susceptibility testing, refer to the manufacturer's instructions for QC **strains** and QC ranges.

General Comments

(1) Refer to Table 1F for antimicrobial agents that should be considered for testing and reporting by microbiology laboratories.

(2) For disk diffusion, test a maximum of 9 disks on a 150-mm plate and 4 disks on a 100-mm plate. For some agents, eg, fluoroquinolones or cephalosporins, only 2 to 3 disks may be tested per plate. Measure the diameter of the zones of complete inhibition (as judged by the unaided eye), including the diameter of the disk. Hold the Petri plate a few inches above a black background illuminated with reflected light. The zone margin should be considered the area showing no obvious, visible growth that can be detected with the unaided eye. Ignore faint growth of tiny colonies that can be detected only with a magnifying lens at the edge of the zone of inhibited growth.

(3) The clinical effectiveness of cefotetan, cefoxitin, and spectinomycin for treating infections due to organisms that produce intermediate results with these agents is unknown.

(4) For disk diffusion testing of *N. gonorrhoeae*, an intermediate result for an antimicrobial agent indicates either a technical problem that should be resolved by repeat testing or a lack of clinical experience in treating infections due to organisms with these zones. Strains with intermediate zones to agents other than cefotetan, cefoxitin, and spectinomycin have a documented lower clinical cure rate (85% to 95%) compared with > 95% for susceptible strains.

Table 2F. *Neisseria gonorrhoeae* (Continued)

(5) The recommended medium for testing *N. gonorrhoeae* consists of GC agar to which a 1% defined growth supplement (1.1 g L-cystine, 0.03 g guanine HCl, 0.003 g thiamine HCl, 0.013 g para-aminobenzoic acid, 0.01 g vitamin B12, 0.1 g cocarboxylase, 0.25 g NAD, 1 g adenine, 10 g L-glutamine, 100 g glucose, 0.02 g ferric nitrate, 25.9 g L-cysteine HCl [in 1 L water]) is added after autoclaving.

NOTE: Information in boldface type is new or modified since the previous edition.

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
		S	I	R	S	I	R	
PENICILLINS								
Penicillin*	10 units	≥ 47	27–46	≤ 26	≤ 0.06	0.12–1	≥ 2	See general comment (4). **(6)** A positive β-lactamase test predicts resistance to penicillin, ampicillin, and amoxicillin. **(7)** A β-lactamase test detects one form of penicillin resistance in *N. gonorrhoeae* and also may be used to provide epidemiological information. Strains with chromosomally mediated resistance can be detected only by the disk diffusion method or the agar dilution MIC method. **(8)** Isolates that produce zones of inhibition ≤ 19 mm around a 10-unit penicillin disk are likely to be β-lactamase–producing strains. However, the β-lactamase test remains preferable to other susceptibility methods for rapid, accurate recognition of this plasmid-mediated penicillin resistance.
CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.)								
Ceftriaxone	30 µg	≥ 35	–	–	≤ 0.25	–	–	
Cefoxitin*	30 µg	≥ 28	24–27	≤ 23	≤ 2	4	≥ 8	See general comment (3).
Cefepime*	30 µg	≥ 31	–	–	≤ 0.5	–	–	
Cefotaxime*	30 µg	≥ 31	–	–	≤ 0.5	–	–	
Cefotetan*	30 µg	≥ 26	20–25	≤ 19	≤ 2	4	≥ 8	See general comment (3).
Ceftizoxime*	30 µg	≥ 38	–	–	≤ 0.5	–	–	

Table 2F. *Neisseria gonorrhoeae* (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
		S	I	R	S	I	R	
CEPHEMS (ORAL)								
Cefixime	5 µg	≥ 31	–	–	≤ 0.25	–	–	
Cefpodoxime*	10 µg	≥ 29	–	–	≤ 0.5	–	–	
MACROLIDES								
Azithromycin	15 µg	≥ 30	–	–	≤ 1	–	–	**(9)** Breakpoint presumes that azithromycin is used in an approved regimen that includes an additional antimicrobial agent.
TETRACYCLINES								
(10) Isolates that **test** susceptible to tetracycline are considered susceptible to doxycycline and minocycline.								
Tetracycline	30 µg	≥ 38	31–37	≤ 30	≤ 0.25	0.5–1	≥ 2	**(11)** Isolates with disk zone diameters ≤ 19 mm usually indicate plasmid-mediated tetracycline resistance. Resistance in these strains should be confirmed by a dilution test (MIC ≥ 16 µg/mL).
FLUOROQUINOLONES								
See general comment (4).								
Ciprofloxacin	5 µg	≥ 41	28–40	≤ 27	≤ 0.06	0.12–0.5	≥ 1	
AMINOCYCLITOLS								
Spectinomycin*	100 µg	≥ 18	15–17	≤ 14	≤ 32	64	≥ 128	See general comment (3).

Abbreviations: CO_2, carbon dioxide; GC, gonococcus (*Neisseria gonorrhoeae*);HCl, hydrochloric acid; I, intermediate; MHB, Mueller-Hinton broth; MIC, minimal inhibitory concentration; NAD, β-nicotinamide adenine dinucleotide; pH, negative logarithm of hydrogen ion concentration; QC, quality control; R, resistant; S, susceptible.

Symbol: *, designation for “Other” agents that are not included in Tables 1 but have established clinical breakpoints.

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Table 2G. Zone Diameter and MIC Breakpoints for *Streptococcus pneumoniae*

Testing Conditions

Medium:	Disk diffusion: MHA with 5% sheep blood or MH-F agar (MHA with 5% mechanically defibrinated horse blood and 20 µg/mL NAD) Broth dilution: CAMHB with LHB (2.5% to 5% v/v) (see CLSI M07[1] for instructions for preparation of LHB) Agar dilution: MHA with sheep blood (5% v/v); recent studies using the agar dilution method have not been performed and reviewed by the subcommittee.
Inoculum:	Colony suspension, equivalent to a 0.5 McFarland standard, prepared using colonies from an overnight (18- to 20-hour) sheep blood agar plate
Incubation:	35°C ± 2°C Disk diffusion: 5% CO_2; 20–24 hours Dilution methods: ambient air; 20–24 hours (CO_2 if necessary, for growth with agar dilution)

QC Recommendations

Refer to the following:

- **Tables 4B and 5B that list acceptable QC ranges applicable for each method**
- **Appendix I to develop a QC plan**

When a commercial test system is used for antimicrobial susceptibility testing, refer to the manufacturer's instructions for QC **strains** and QC ranges.

Refer to Table 3J for additional testing recommendations, reporting suggestions, and QC.

General Comments

(1) Refer to Table 1G for antimicrobial agents that should be considered for testing and reporting by microbiology laboratories.

(2) For disk diffusion, test a maximum of 9 disks on a 150-mm plate and 4 disks on a 100-mm plate. Measure the diameter of the zones of complete inhibition (as judged by the unaided eye), including the diameter of the disk (see CLSI M02QG[2]). The zone margin should be considered the area showing no obvious, visible growth that can be detected with the unaided eye. Do not measure the zone of inhibition of hemolysis. Measure the zones from the upper surface of the agar illuminated with reflected light, with the cover removed. Ignore faint growth of tiny colonies that can be detected only with a magnifying lens at the edge of the zone of inhibited growth. With trimethoprim and the sulfonamides, antagonists in the medium may allow some slight growth; therefore, disregard slight growth (20% or less of the lawn of growth) and measure the more obvious margin to determine the zone diameter.

Table 2G. *Streptococcus pneumoniae* (Continued)

(3) For pneumococci when testing chloramphenicol, clindamycin, erythromycin, linezolid, tedizolid, and tetracycline by broth microdilution MIC, trailing growth can make end-point determination difficult. In such cases, read the MIC at the lowest concentration where the trailing begins. Tiny buttons of growth should be ignored (see CLSI M07[1]). With trimethoprim and the sulfonamides, antagonists in the medium may allow some slight growth; therefore, read the end point at the concentration in which there is ≥ 80% reduction in growth compared with the control (see CLSI M07[1]).

(4) Amoxicillin, ampicillin, cefepime, cefotaxime, ceftriaxone, cefuroxime, ertapenem, imipenem, and meropenem may be used to treat pneumococcal infections; however, reliable disk diffusion susceptibility tests with these agents do not yet exist. The *in vitro* activity of these agents is best determined using an MIC method.

(5) Penicillin and cefotaxime, ceftriaxone, or meropenem should be tested by a reliable MIC method (such as that described in CLSI M07[1]) and reported routinely with *S. pneumoniae* isolated from CSF. Such isolates can also be tested against vancomycin using the MIC or disk diffusion method. With isolates from other sites, the oxacillin disk test may be used. If the oxacillin zone size is ≤ 19 mm, cefotaxime, ceftriaxone, meropenem, or penicillin MICs should be determined.

(6) For disk diffusion, results using MHA with 5% sheep blood and MH-F agar were equivalent when disk contents, testing conditions, and zone diameter breakpoints in this table were used. Disk diffusion QC ranges for *S. pneumoniae* ATCC®[a] 49619 in Table 4B apply to testing using either MHA with 5% sheep blood or MH-F agar.

NOTE: Information in boldface type is new or modified since the previous edition.

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
		S	I	R	S	I	R	
PENICILLINS								
(7) For nonmeningitis isolates, a penicillin MIC of ≤ 0.06 µg/mL (or oxacillin zone ≥ 20 mm) can predict susceptibility to the following β-lactams: ampicillin (oral or parenteral), ampicillin-sulbactam, amoxicillin, amoxicillin-clavulanate, cefaclor, cefdinir, cefditoren, cefepime, cefotaxime, cefpodoxime, cefprozil, ceftaroline, ceftizoxime, ceftriaxone, cefuroxime, doripenem, ertapenem, imipenem, loracarbef, meropenem. See general comment (5).								
Penicillin	1 µg oxacillin	≥ 20	–	–	–	–	–	**(8)** Isolates of pneumococci with oxacillin zone sizes ≥ 20 mm are susceptible (MIC ≤ 0.06 µg/mL) to penicillin. Penicillin and cefotaxime, ceftriaxone, or meropenem MICs should be determined for isolates with oxacillin zone diameters ≤ 19 mm, because zones ≤ 19 mm occur with penicillin-resistant, -intermediate, or certain -susceptible strains. For isolates with oxacillin zones ≤ 19 mm, do not report penicillin as resistant without performing a penicillin MIC test.

Table 2G. *Streptococcus pneumoniae* (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
		S	I	R	S	I	R	
PENICILLINS (Continued)								
Penicillin parenteral (nonmeningitis)	–	–	–	–	≤ 2	4	≥ 8	**(9)** For all isolates other than those from CSF, report interpretations for both meningitis and nonmeningitis.
Penicillin parenteral (meningitis)	–	–	–	–	≤ 0.06	–	≥ 0.12	**(10)** For CSF isolates, report only meningitis interpretations. See general comment (5).
Penicillin (oral penicillin V)	–	–	–	–	≤ 0.06	0.12–1	≥ 2	**(11)** Interpretations for oral penicillin may be reported for isolates other than those from CSF.
Amoxicillin (nonmeningitis) Amoxicillin-clavulanate (nonmeningitis)	–	–	–	–	≤ 2 ≤ 2/1	4 4/2	≥ 8 ≥ 8/4	
CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.)								
See comment (7).								
Cefepime (meningitis)*	–	–	–	–	≤ 0.5	1	≥ 2	**(12)** In the United States, for CSF isolates, report only nonmeningitis interpretations. There is not an FDA-approved indication for the use of cefepime for meningitis in the United States.
Cefepime (nonmeningitis)	–	–	–	–	≤ 1	2	≥ 4	**(13)** In the United States, report only interpretations for nonmeningitis and include the nonmeningitis notation on the report.
Cefotaxime (meningitis) Ceftriaxone (meningitis)	– –	– –	– –	– –	≤ 0.5 ≤ 0.5	1 1	≥ 2 ≥ 2	**(14)** For CSF isolates, report only meningitis interpretations. **(15)** ***Rx:*** Use of cefotaxime or ceftriaxone in meningitis requires therapy with maximum doses. See general comment (5).
Cefotaxime (nonmeningitis) Ceftriaxone (nonmeningitis)	– –	– –	– –	– –	≤ 1 ≤ 1	2 2	≥ 4 ≥ 4	**(16)** For all isolates other than those from CSF, report interpretations for both meningitis and nonmeningitis.

Table 2G. *Streptococcus pneumoniae* (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
		S	I	R	S	I	R	
CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.) (Continued)								
Ceftaroline (nonmeningitis)	30 µg	≥ 26	–	–	≤ 0.5	–	–	
Cefuroxime (parenteral)	–	–	–	–	≤ 0.5	1	≥ 2	
CEPHEMS (ORAL)								
See comment (7).								
Cefuroxime (oral)	–	–	–	–	≤ 1	2	≥ 4	**(17)** Interpretations for oral cefuroxime may be reported for isolates other than those from CSF.
Cefaclor*	–	–	–	–	≤ 1	2	≥ 4	
Cefdinir*	–	–	–	–	≤ 0.5	1	≥ 2	
Cefpodoxime*	–	–	–	–	≤ 0.5	1	≥ 2	
Cefprozil*	–	–	–	–	≤ 2	4	≥ 8	
Loracarbef*	–	–	–	–	≤ 2	4	≥ 8	
CARBAPENEMS								
See comment (7).								
Meropenem	–	–	–	–	≤ 0.25	0.5	≥ 1	See general comment (5) and comment (8).
Ertapenem	–	–	–	–	≤ 1	2	≥ 4	
Imipenem	–	–	–	–	≤ 0.12	0.25–0.5	≥ 1	
Doripenem*	–	–	–	–	≤ 1	–	–	
GLYCOPEPTIDES								
Vancomycin	30 µg	≥ 17	–	–	≤ 1	–	–	See general comment (5).
MACROLIDES								
(18) Susceptibility and resistance to azithromycin, clarithromycin, and dirithromycin can be predicted by testing erythromycin. **(19)** Not routinely reported on organisms isolated from the urinary tract.								
Erythromycin	15 µg	≥ 21	16–20	≤ 15	≤ 0.25	0.5	≥ 1	
Azithromycin*	15 µg	≥ 18	14–17	≤ 13	≤ 0.5	1	≥ 2	
Clarithromycin*	15 µg	≥ 21	17–20	≤ 16	≤ 0.25	0.5	≥ 1	
Dirithromycin*	15 µg	≥ 18	14–17	≤ 13	≤ 0.5	1	≥ 2	

Table 2G. *Streptococcus pneumoniae* (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
		S	I	R	S	I	R	
TETRACYCLINES								
(20) Isolates that **test** susceptible to tetracycline are considered susceptible to doxycycline. **Isolates that test intermediate or resistant to tetracycline should be tested against doxycycline if that result is needed for treatment.**								
Tetracycline	30 µg	≥ 28	25–27	≤ 24	≤ 1	2	≥ 4	
Doxycycline	30 µg	≥ 28	25–27	≤ 24	≤ 0.25	0.5	≥ 1	
FLUOROQUINOLONES								
Gemifloxacin*	5 µg	≥ 23	20–22	≤ 19	≤ 0.12	0.25	≥ 0.5	**(21)** Organisms that are susceptible to levofloxacin are also considered susceptible to gemifloxacin and moxifloxacin. However, some organisms that are intermediate or resistant to levofloxacin may be susceptible to gemifloxacin, moxifloxacin, or both.
Levofloxacin	5 µg	≥ 17	14–16	≤ 13	≤ 2	4	≥ 8	
Moxifloxacin	5 µg	≥ 18	15–17	≤ 14	≤ 1	2	≥ 4	
Gatifloxacin*	5 µg	≥ 21	18–20	≤ 17	≤ 1	2	≥ 4	
Ofloxacin*	5 µg	≥ 16	13–15	≤ 12	≤ 2	4	≥ 8	
Sparfloxacin*	5 µg	≥ 19	16–18	≤ 15	≤ 0.5	1	≥ 2	
FOLATE PATHWAY ANTAGONISTS								
Trimethoprim-sulfamethoxazole	1.25/ 23.75 µg	≥ 19	16–18	≤ 15	≤ 0.5/9.5	1/19–2/38	≥ 4/76	
PHENICOLS								
Chloramphenicol*	30 µg	≥ 21	–	≤ 20	≤ 4	–	≥ 8	See comment (19).
ANSAMYCINS								
Rifampin	5 µg	≥ 19	17–18	≤ 16	≤ 1	2	≥ 4	**(22)** ***Rx:*** Rifampin should not be used alone for antimicrobial therapy.
LINCOSAMIDES								
Clindamycin	2 µg	≥ 19	16–18	≤ 15	≤ 0.25	0.5	≥ 1	**(23)** For isolates that test erythromycin resistant and clindamycin susceptible or intermediate, testing for ICR by disk diffusion using the D-zone test or by broth microdilution is required before reporting clindamycin (see Table 3J, CLSI M02,[3] and CLSI M07[1]). See comment (19).

Table 2G. *Streptococcus pneumoniae* (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
		S	I	R	S	I	R	
STREPTOGRAMINS								
Quinupristin-dalfopristin*	15 µg	≥ 19	16–18	≤ 15	≤ 1	2	≥ 4	
OXAZOLIDINONES								
Linezolid	30 µg	≥ 21	–	–	≤ 2	–	–	
PLEUROMUTILINS								
Lefamulin	20 µg	≥ 19	–		≤ 0.5	–	–	See comment (19).

Abbreviations: ATCC®, American Type Culture Collection; CAMHB, cation-adjusted Mueller-Hinton broth; CO_2, carbon dioxide; CSF, cerebrospinal fluid; FDA, US Food and Drug Administration; I, intermediate; ICR, inducible clindamycin resistance; LHB, lysed horse blood; MHA, Mueller-Hinton agar; MH-F, Mueller-Hinton fastidious; MIC, minimal inhibitory concentration; NAD, β-nicotinamide adenine dinucleotide; QC, quality control; R, resistant; S, susceptible.
Symbol: *, designation for "Other" agents that are not included in Tables 1 but have established clinical breakpoints.

Footnote

a. ATCC® is a registered trademark of the American Type Culture Collection.

References for Table 2G

[1] CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*. 12th ed. CLSI standard M07. Clinical and Laboratory Standards Institute; 2024.

[2] CLSI. *M02 Disk Diffusion Reading Guide*. 2nd ed. CLSI quick guide M02QG. Clinical and Laboratory Standards Institute; 2024.

[3] CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests*. 14th ed. CLSI standard M02. Clinical and Laboratory Standards Institute; 2024.

Table 2H-1. Zone Diameter and MIC Breakpoints for *Streptococcus* spp. β-Hemolytic Group

Testing Conditions

Medium: Disk diffusion: MHA with 5% sheep blood
Broth dilution: CAMHB with LHB (2.5% to 5% v/v); the CAMHB should be supplemented to 50 µg/mL calcium for daptomycin (see CLSI M07[1] for instructions for preparation of LHB).
Agar dilution: MHA with sheep blood (5% v/v); recent studies using the agar dilution method have not been performed and reviewed by the subcommittee.

Inoculum: Colony suspension, equivalent to a 0.5 McFarland standard, using colonies from an overnight (18- to 20-hour) sheep blood agar plate

Incubation: 35°C ± 2°C
Disk diffusion: 5% CO_2; 20–24 hours
Dilution methods: ambient air; 20–24 hours (CO_2 if necessary, for growth with agar dilution)

QC Recommendations

Refer to the following:

- **Tables 4B and 5B that list acceptable QC ranges applicable for each method**
- **Appendix I to develop a QC plan**

When a commercial test system is used for antimicrobial susceptibility testing, refer to the manufacturer's instructions for QC **strains** and QC ranges.

Refer to Table 3J for additional testing recommendations, reporting suggestions, and QC.

General Comments

(1) Refer to Table 1H-1 for antimicrobial agents that should be considered for testing and reporting by microbiology laboratories.

(2) For disk diffusion, test a maximum of 9 disks on a 150-mm plate and 4 disks on a 100-mm plate. Measure the diameter of the zones of complete inhibition (as judged by the unaided eye), including the diameter of the disk (see CLSI M02QG[2]). The zone margin should be considered the area showing no obvious, visible growth that can be detected with the unaided eye. Do not measure the zone of inhibition of hemolysis. Measure the zones from the upper surface of the agar illuminated with reflected light, with the cover removed. Ignore faint growth of tiny colonies that can be detected only with a magnifying lens at the edge of the zone of inhibited growth.

(3) For β-hemolytic streptococci when testing chloramphenicol, clindamycin, erythromycin, linezolid, tedizolid, and tetracycline by broth microdilution MIC, trailing growth can make end-point determination difficult. In such cases, read the MIC at the lowest concentration where the trailing begins. Tiny buttons of growth should be ignored (see CLSI M07[1]).

Table 2H-1. *Streptococcus* spp. β-Hemolytic Group (Continued)

(4) For this table, the β-hemolytic group includes the large colony–forming pyogenic strains of streptococci with group A (*S. pyogenes*), C, or G antigens and strains with Group B (*S. agalactiae*) antigen. Small colony–forming β-hemolytic strains with group A, C, F, or G antigens (*S. anginosus* group, previously *S. milleri*) are considered part of the viridans group, and breakpoints for the viridans group should be used (see Table 2H-2).

(5) Penicillin and ampicillin are drugs of choice for treating β-hemolytic streptococcal infections. Susceptibility testing of penicillins and other β-lactams approved by the FDA for treatment of β-hemolytic streptococcal infections does not need to be performed routinely, because nonsusceptible isolates (ie, penicillin MICs > 0.12 and ampicillin MICs > 0.25 µg/mL) are extremely rare in any β-hemolytic streptococci and have not been reported for *S. pyogenes*. If testing is performed, any β-hemolytic streptococcal isolate found to be nonsusceptible should be re-identified, retested, and, if confirmed, submitted to a public health laboratory. See Appendix A for additional instructions.

(6) Breakpoints for *Streptococcus* spp. β-hemolytic group are proposed based on population distributions of various species, pharmacokinetics of the antimicrobial agents, previously published literature, and the clinical experience of subcommittee members. Systematically collected clinical data were not available for review with many of the antimicrobial agents in this table.

NOTE: Information in boldface type is new or modified since the previous edition.

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
		S	I	R	S	I	R	
PENICILLINS								
(7) An organism that is susceptible to penicillin can be considered susceptible to antimicrobial agents listed here when used for approved indications and does not need to be tested against those agents. For groups A, B, C, and G β-hemolytic streptococci, penicillin is tested as a surrogate for ampicillin, amoxicillin, amoxicillin-clavulanate, ampicillin-sulbactam, cefazolin, cefepime, ceftaroline, cephradine, cephalothin, cefotaxime, ceftriaxone, ceftizoxime, imipenem, ertapenem, and meropenem. For group A β-hemolytic streptococci, penicillin is also a surrogate for cefaclor, cefdinir, cefprozil, ceftibuten, cefuroxime, and cefpodoxime.								
Penicillin or	10 units	≥ 24	–	–	≤ 0.12	–	–	See general comment (5).
ampicillin	10 µg	≥ 24	–	–	≤ 0.25	–	–	
CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.)								
See comment (7).								
Cefepime or	30 µg	≥ 24	–	–	≤ 0.5	–	–	
cefotaxime or	30 µg	≥ 24	–	–	≤ 0.5	–	–	
ceftriaxone	30 µg	≥ 24	–	–	≤ 0.5	–	–	
Ceftaroline	30 µg	≥ 26	–	–	≤ 0.5	–	–	

Table 2H-1. *Streptococcus* spp. β-Hemolytic Group (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm S	I	R	Interpretive Categories and MIC Breakpoints, µg/mL S	I	R	Comments
CARBAPENEMS								
See comment (7).								
Doripenem*	–	–	–	–	≤ 0.12	–	–	
Ertapenem*	–	–	–	–	≤ 1	–	–	
Meropenem*	–	–	–	–	≤ 0.5	–	–	
GLYCOPEPTIDES								
Vancomycin	30 µg	≥ 17	–	–	≤ 1	–	–	
LIPOGLYCOPEPTIDES								
Dalbavancin	–	–	–	–	≤ 0.25	–	–	**(8)** Report only on *S. pyogenes*, *S. agalactiae*, and *S. dysgalactiae*.
Oritavancin	–	–	–	–	≤ 0.25	–	–	
Telavancin	–	–	–	–	≤ 0.12	–	–	
LIPOPEPTIDES								
Daptomycin	–	–	–	–	≤ 1	–	–	**(9)** Not routinely reported on organisms isolated from the lower respiratory tract.
MACROLIDES								
(10) Susceptibility and resistance to azithromycin, clarithromycin, and dirithromycin can be predicted by testing erythromycin. **(11)** Not routinely reported on organisms isolated from the urinary tract.								
Erythromycin	15 µg	≥ 21	16–20	≤ 15	≤ 0.25	0.5	≥ 1	**(12)** ***Rx:*** Recommendations for intrapartum prophylaxis for group B streptococci are penicillin or ampicillin. Although cefazolin is recommended for penicillin-allergic women at low risk for anaphylaxis, those at high risk for anaphylaxis may receive clindamycin or vancomycin (if the isolate is not susceptible to clindamycin).^{3} Group B streptococci are susceptible to ampicillin, penicillin, and cefazolin but may be resistant to erythromycin and clindamycin. When clindamycin is being considered for intrapartum prophylaxis (eg, pregnant woman with severe penicillin allergy), erythromycin and clindamycin (including ICR) should be tested, but only clindamycin should be reported. See Table 3J.

Table 2H-1. *Streptococcus* spp. β-Hemolytic Group (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
		S	I	R	S	I	R	
MACROLIDES (Continued)								
Azithromycin*	15 µg	≥ 18	14–17	≤ 13	≤ 0.5	1	≥ 2	
Clarithromycin*	15 µg	≥ 21	17–20	≤ 16	≤ 0.25	0.5	≥ 1	
Dirithromycin*	15 µg	≥ 18	14–17	≤ 13	≤ 0.5	1	≥ 2	
TETRACYCLINES								
(13) Isolates that **test** susceptible to tetracycline are considered susceptible to doxycycline and minocycline.								
Tetracycline	30 µg	≥ 23	19–22	≤ 18	≤ 2	4	≥ 8	
FLUOROQUINOLONES								
Levofloxacin	5 µg	≥ 17	14–16	≤ 13	≤ 2	4	≥ 8	
Gatifloxacin*	5 µg	≥ 21	18–20	≤ 17	≤ 1	2	≥ 4	
Grepafloxacin*	5 µg	≥ 19	16–18	≤ 15	≤ 0.5	1	≥ 2	
Ofloxacin*	5 µg	≥ 16	13–15	≤ 12	≤ 2	4	≥ 8	
Trovafloxacin*	10 µg	≥ 19	16–18	≤ 15	≤ 1	2	≥ 4	
PHENICOLS								
Chloramphenicol*	30 µg	≥ 21	18–20	≤ 17	≤ 4	8	≥ 16	See comment (11).
LINCOSAMIDES								
Clindamycin	2 µg	≥ 19	16–18	≤ 15	≤ 0.25	0.5	≥ 1	See comments (11) and (12). **(14)** For isolates that test erythromycin resistant and clindamycin susceptible or intermediate, testing for ICR by disk diffusion using the D-zone test or by broth microdilution is required before reporting clindamycin. See Table 3J, CLSI M02,[4] and CLSI M07.[1]
STREPTOGRAMINS								
Quinupristin-dalfopristin*	15 µg	≥ 19	16–18	≤ 15	≤ 1	2	≥ 4	**(15)** Report only on *S. pyogenes*.

Table 2H-1. *Streptococcus* spp. β-Hemolytic Group (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
		S	I	R	S	I	R	
OXAZOLIDINONES								
(16) *S. agalactiae* and *S. pyogenes* that test susceptible to linezolid are considered susceptible to tedizolid. Isolates that **test** nonsusceptible to linezolid **should be tested against tedizolid if that result is needed for treatment.**								
Linezolid	30 µg	≥ 21	–	–	≤ 2	–	–	
Tedizolid	2 µg	≥ 15	–	–	≤ 0.5	–	–	**(17)** Report only on *S. pyogenes* and *S. agalactiae*.

Abbreviations: CAMHB, cation-adjusted Mueller-Hinton broth; CO_2, carbon dioxide; FDA, US Food and Drug Administration; I, intermediate; ICR, inducible clindamycin resistance; LHB, lysed horse blood; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; QC, quality control; R, resistant; S, susceptible.
Symbol: *, designation for "Other" agents not included in Tables 1 but have established clinical breakpoints.

References for Table 2H-1

[1] CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically.* 12th ed. CLSI standard M07. Clinical and Laboratory Standards Institute; 2024.

[2] CLSI. *M02 Disk Diffusion Reading Guide.* 2nd ed. CLSI quick guide M02-Ed14-QG. Clinical and Laboratory Standards Institute; 2024.

[3] American College of Obstetricians and Gynecologists. Prevention of group B streptococcal early-onset disease in newborns: ACOG Committee Opinion, Number 797. *Obstet Gynecol.* 2020;135(2):e51-e72. doi:10.1097/AOG.0000000000003668

[4] CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests.* 14th ed. CLSI standard M02. Clinical and Laboratory Standards Institute; 2024.

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Table 2H-2. Zone Diameter and MIC Breakpoints for *Streptococcus* spp. Viridans Group

Testing Conditions

Medium:	Disk diffusion: MHA with 5% sheep blood Broth dilution: CAMHB with LHB (2.5% to 5% v/v); the CAMHB should be supplemented to 50 µg/mL calcium for daptomycin (see CLSI M07[1] for instructions for preparation of LHB). Agar dilution: MHA with sheep blood (5% v/v); recent studies using the agar dilution method have not been performed and reviewed by the subcommittee
Inoculum:	Colony suspension, equivalent to a 0.5 McFarland standard using colonies from an overnight (18- to 20-hour) sheep blood agar plate
Incubation:	35°C ± 2°C Disk diffusion: 5% CO_2; 20–24 hours Dilution methods: ambient air; 20–24 hours (CO_2 if necessary, for growth with agar dilution)

QC Recommendations

Refer to the following:

- **Tables 4B and 5B that list acceptable QC ranges applicable for each method**
- **Appendix I to develop a QC plan**

When a commercial test system is used for antimicrobial susceptibility testing, refer to the manufacturer's instructions for QC **strains** and QC ranges.

General Comments

(1) Refer to Table 1H-2 for antimicrobial agents that should be considered for testing and reporting by microbiology laboratories.

(2) For disk diffusion, measure the diameter of the zones of complete inhibition (as judged by the unaided eye), including the diameter of the disk. The zone margin should be considered the area showing no obvious, visible growth that can be detected with the unaided eye. Do not measure the zone of inhibition of hemolysis. Measure the zones from the upper surface of the agar illuminated with reflected light, with the cover removed. Ignore faint growth of tiny colonies that can be detected only with a magnifying lens at the edge of the zone of inhibited growth.

(3) For viridans streptococci when testing chloramphenicol, clindamycin, erythromycin, linezolid, tedizolid, and tetracycline by broth microdilution MIC, trailing growth can make end point determination difficult. In such cases, read the MIC at the lowest concentration where the trailing begins. Tiny buttons of growth should be ignored (see CLSI M07[1]).

(4) The viridans group of streptococci includes the following five groups, with several species within each group: *S. mutans* group, *S. salivarius* group, *S. bovis* group, *S. anginosus* group (previously *S. milleri* group), and *S. mitis* group. The *S. anginosus* group includes small colony–forming β-hemolytic strains with groups A, C, F, and G antigens. For detailed information on the species within the groups, please refer to recent literature.

Table 2H-2. *Streptococcus* spp. Viridans Group (Continued)

(5) Breakpoints for *Streptococcus* spp. viridans group are proposed based on population distributions of various species, pharmacokinetics of the antimicrobial agents, previously published literature, and the clinical experience of subcommittee members. Systematically collected clinical data were not available for review with many of the antimicrobial agents in this table.

NOTE: Information in boldface type is new or modified since the previous edition.

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
		S	I	R	S	I	R	
PENICILLINS								
Penicillin	–	–	–	–	≤ 0.12	0.25–2	≥ 4	**(6)** Viridans streptococci isolated from normally sterile anatomical sites (eg, CSF, blood, bone) should be tested for penicillin susceptibility using an MIC method. **(7)** A penicillin MIC of ≤ 0.125 µg/mL is the same as a penicillin MIC of ≤ 0.12 µg/mL and both should be interpreted as susceptible. Laboratories should report an MIC of ≤ 0.125 µg/mL as ≤ 0.12 µg/mL. **(8)** ***Rx:*** Penicillin- or ampicillin-intermediate isolates may necessitate combined therapy with an aminoglycoside for bactericidal action.
Ampicillin					≤ 0.25	0.5–4	≥ 8	
β-LACTAM COMBINATION AGENTS								
Ceftolozane-tazobactam	–	–	–	–	≤ 8/4	16/4	≥ 32/4	
CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.)								
Cefepime	30 µg	≥ 24	22–23	≤ 21	≤ 1	2	≥ 4	
Cefotaxime	30 µg	≥ 28	26–27	≤ 25	≤ 1	2	≥ 4	
Ceftriaxone	30 µg	≥ 27	25–26	≤ 24	≤ 1	2	≥ 4	
CARBAPENEMS								
Doripenem*	–	–	–	–	≤ 1	–	–	
Ertapenem*	–	–	–	–	≤ 1	–	–	
Meropenem*	–	–	–	–	≤ 0.5	–	–	
GLYCOPEPTIDES								
Vancomycin	30 µg	≥ 17	–	–	≤ 1	–	–	

Table 2H-2. *Streptococcus* spp. Viridans Group (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
		S	I	R	S	I	R	
LIPOGLYCOPEPTIDES								
Dalbavancin	–	–	–	–	≤ 0.25	–	–	**(9)** Report only on *S. anginosus* group (including *S. anginosus*, *S. intermedius*, and *S. constellatus*).
Oritavancin	–	–	–	–	≤ 0.25	–	–	
Telavancin	–	–	–	–	≤ 0.06	–	–	
LIPOPEPTIDES								
Daptomycin*	–	–	–	–	≤ 1	–	–	**(10)** Not routinely reported on organisms isolated from the lower respiratory tract.
MACROLIDES								
(11) Susceptibility and resistance to azithromycin, clarithromycin, and dirithromycin can be predicted by testing erythromycin. **(12)** Not routinely reported on organisms isolated from the urinary tract.								
Erythromycin	15 µg	≥ 21	16–20	≤ 15	≤ 0.25	0.5	≥ 1	
Azithromycin*	15 µg	≥ 18	14–17	≤ 13	≤ 0.5	1	≥ 2	
Clarithromycin*	15 µg	≥ 21	17–20	≤ 16	≤ 0.25	0.5	≥ 1	
Dirithromycin*	15 µg	≥ 18	14–17	≤ 13	≤ 0.5	1	≥ 2	
TETRACYCLINES								
(13) Isolates that **test** susceptible to tetracycline are considered susceptible to doxycycline and minocycline.								
Tetracycline*	30 µg	≥ 23	19–22	≤ 18	≤ 2	4	≥ 8	
FLUOROQUINOLONES								
Levofloxacin	5 µg	≥ 17	14–16	≤ 13	≤ 2	4	≥ 8	
Ofloxacin*	5 µg	≥ 16	13–15	≤ 12	≤ 2	4	≥ 8	
Gatifloxacin*	5 µg	≥ 21	18–20	≤ 17	≤ 1	2	≥ 4	
Grepafloxacin*	5 µg	≥ 19	16–18	≤ 15	≤ 0.5	1	≥ 2	
Trovafloxacin*	10 µg	≥ 19	16–18	≤ 15	≤ 1	2	≥ 4	
PHENICOLS								
Chloramphenicol*	30 µg	≥ 21	18–20	≤ 17	≤ 4	8	≥ 16	See comment (12).

Table 2H-2. *Streptococcus* spp. Viridans Group (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
		S	I	R	S	I	R	
LINCOSAMIDES								
Clindamycin	2 µg	≥ 19	16–18	≤ 15	≤ 0.25	0.5	≥ 1	See comment (12).
STREPTOGRAMINS								
Quinupristin-dalfopristin*	15 µg	≥ 19	16–18	≤ 15	≤ 1	2	≥ 4	
OXAZOLIDINONES								
(14) *S. anginosus* group that test susceptible to linezolid **are** considered susceptible to tedizolid. **Isolates** that **test** nonsusceptible to linezolid **should be tested against tedizolid if that result is needed for treatment.**								
Linezolid	30 µg	≥ 21	–	–	≤ 2	–	–	
Tedizolid	2 µg	≥ 18	–	–	≤ 0.25	–	–	See comment (9).

Abbreviations: CAMHB, cation-adjusted Mueller-Hinton broth; CO_2, carbon dioxide; CSF, cerebrospinal fluid; I, intermediate; LHB, lysed horse blood; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; QC, quality control; R, resistant; S, susceptible.

Symbol: *, designation for “Other” agents that are not included in Tables 1 but have established clinical breakpoints.

Reference for Table 2H-2

1 CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically.* 12th ed. CLSI standard M07. Clinical and Laboratory Standards Institute; 2024.

Table 2I. Zone Diameter and MIC Breakpoints for *Neisseria meningitidis*

Testing Conditions

Medium: Disk diffusion: MHA with 5% sheep blood
Broth microdilution: CAMHB supplemented with LHB (2.5% to 5% v/v) (see CLSI M07[1] for preparation of LHB)
Agar dilution: MHA supplemented with sheep blood (5% v/v)

Inoculum: Colony suspension from 20–24 hours growth from chocolate agar incubated at 35°C; 5% CO_2; equivalent to a 0.5 McFarland standard. Colonies grown on sheep blood agar may be used for inoculum preparation. However, the 0.5 McFarland suspension obtained from sheep blood agar will contain approximately 50% fewer CFU/mL. This must be considered when preparing the final dilution before panel inoculation, as guided by colony counts.

Incubation: 35°C ± 2°C; 5% CO_2; 20–24 hours

QC Recommendations

Refer to the following:

- **Tables 4A-1, 4B, 5A-1, and 5B that list acceptable QC ranges applicable for each method**
- **Appendix I to develop a QC plan**

When a commercial test system is used for antimicrobial susceptibility testing, refer to the manufacturer's instructions for QC **strains** and QC ranges.

General Comments

(1) Refer to Table 1I for antimicrobial agents that should be considered for testing and reporting by microbiology laboratories.

Important: For complete information on safety precautions, see *Biosafety in Microbiological and Biomedical Laboratories*. 6th ed. Centers for Disease Control and Prevention; 2020. Accessed 15 October 2024. https://www.cdc.gov/labs/pdf/SF__19_308133-A_BMBL6_00-BOOK-WEB-final-3.pdf

(2) **Recommended precautions:** Perform all AST of *N. meningitidis* in a BSC. Manipulating *N. meningitidis* outside a BSC is associated with increased risk for contracting meningococcal disease. Laboratory-acquired meningococcal disease is associated with a case fatality rate of 50%. Exposure to droplets or aerosols of *N. meningitidis* is the most likely risk for laboratory-acquired infection. Rigorous protection from droplets or aerosols is mandated when microbiological procedures (including AST) are performed on all *N. meningitidis* isolates.

(3) If a BSC is unavailable, manipulation of these isolates should be minimized, limited to Gram staining or serogroup identification using phenolized saline solution, while wearing a laboratory coat and gloves and working behind a full face splash shield. Use BSL-3 practices, procedures, and containment equipment for activities with a high potential for droplet or aerosol production and for activities involving production quantities or high concentrations of infectious materials. If BSL-2 or BSL-3 facilities are not available, forward isolates to a referral or public health laboratory with a minimum of BSL-2 facilities.

Table 2I. *Neisseria meningitidis* (Continued)

(4) Laboratorians who are exposed routinely to potential aerosols of *N. meningitidis* should consider vaccination according to the current recommendations of the Centers for Disease Control and Prevention Advisory Committee on Immunization Practices. Accessed 29 October 2024. https://www.cdc.gov/acip-recs/hcp/vaccine-specific/index.html

(5) For disk diffusion, test a maximum of 5 disks on a 150-mm plate and 2 disks on a 100-mm plate. Measure the diameter of the zones of complete inhibition (as judged by the unaided eye), including the diameter of the disk. Measure the zones from the upper surface of the agar illuminated with reflected light, with the cover removed. Ignore faint growth of tiny colonies that can be detected only with a magnifying lens at the edge of the zone of inhibited growth. With trimethoprim and the sulfonamides, antagonists in the medium may allow some slight growth; therefore, disregard slight growth (20% or less of the lawn of growth) and measure the more obvious margin to determine the zone diameter.

(6) Breakpoints are based on population distributions of MICs of various agents, pharmacokinetics of the agents, previously published literature, and the clinical experience of subcommittee members. Systematically collected clinical data were not available to review with many of the antimicrobial agents in this table.

(7) With azithromycin, breakpoints were developed initially using MICs determined by incubation in ambient air for the pharmacodynamic calculations.

NOTE: Information in boldface type is new or modified since the previous edition.

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
		S	I	R	S	I	R	
PENICILLINS								
Penicillin	–	–	–	–	≤ 0.06	0.12–0.25	≥ 0.5	
Ampicillin*	–	–	–	–	≤ 0.12	0.25–1	≥ 2	
CEPHEMS								
Cefotaxime or	30 µg	≥ 34	–	–	≤ 0.12	–	–	
ceftriaxone	30 µg	≥ 34	–	–	≤ 0.12	–	–	
CARBAPENEMS								
Meropenem	10 µg	≥ 30	–	–	≤ 0.25	–	–	
MACROLIDES								
Azithromycin	15 µg	≥ 20	–	–	≤ 2	–	–	See general comment (7). **(8)** May be appropriate only for prophylaxis of meningococcal case contacts. These breakpoints do not apply to therapy of patients with invasive meningococcal disease.

Table 2I. *Neisseria meningitidis* (Continued)

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
		S	I	R	S	I	R	
TETRACYCLINES								
Minocycline	30 µg	≥ 26	–	–	≤ 2	–	–	See comment (8).
FLUOROQUINOLONES								
(9) For surveillance purposes, a nalidixic acid MIC ≥ 8 µg/mL or a zone ≤ 25 mm may correlate with diminished fluoroquinolone susceptibility.								
Ciprofloxacin	5 µg	≥ 35	33–34	≤ 32	≤ 0.03	0.06	≥ 0.12	See comment (8).
Levofloxacin	–	–	–	–	≤ 0.03	0.06	≥ 0.12	
FOLATE PATHWAY ANTAGONISTS								
Trimethoprim-sulfamethoxazole	1.25/ 23.75 µg	≥ 30	26–29	≤ 25	≤ 0.12/ 2.4	0.25/4.75	≥ 0.5/ 9.5	**(10)** Trimethoprim-sulfamethoxazole is the preferred disk for detection of sulfonamide resistance. Trimethoprim-sulfamethoxazole testing predicts susceptibility and resistance to trimethoprim-sulfamethoxazole and sulfonamides. Sulfonamides may be appropriate only for prophylaxis of meningococcal case contacts.
PHENICOLS								
Chloramphenicol*	30 µg	≥ 26	20–25	≤ 19	≤ 2	4	≥ 8	**(11)** Not routinely reported on organisms isolated from the urinary tract.
ANSAMYCINS								
Rifampin	5 µg	≥ 25	20–24	≤ 19	≤ 0.5	1	≥ 2	See comment (8).

Abbreviations: AST, antimicrobial susceptibility testing; BSC, biological safety cabinet; BSL-2, biosafety level 2; BSL-3, biosafety level 3; CAMHB, cation-adjusted Mueller-Hinton broth; CFU, colony-forming unit(s); CO_2, carbon dioxide; I, intermediate; LHB, lysed horse blood; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; QC, quality control; R, resistant; S, susceptible.

Symbol: *, designation for “Other” agents not included in Tables 1 but have established clinical breakpoints.

Reference for Table 2I

[1] CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically.* 12th ed. CLSI standard M07. Clinical and Laboratory Standards Institute; 2024.

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Table 2J. MIC Breakpoints for Anaerobes

Testing Conditions

Medium:	Agar dilution (for all anaerobes): Brucella agar supplemented with hemin (5 µg/mL), vitamin K_1 (1 µg/mL), and laked sheep blood (5% v/v) Broth microdilution (for ***Bacteroides fragilis* and *Bacteroides thetaiotaomicron*** only): Brucella broth supplemented with hemin (5 µg/mL), vitamin K_1 (1 µg/mL), and LHB (5% v/v)
Inoculum:	Broth culture method or colony suspension, equivalent to 0.5 McFarland suspension Agar: 10^5 CFU per spot Broth: 10^6 CFU/mL
Incubation:	36°C ± 1°C, anaerobically Broth microdilution: 46–48 hours Agar dilution: 42–48 hours

QC Recommendations

Refer to the following:

- **Tables 5D and 5E that list acceptable QC ranges applicable for each method**
- **Appendix I to develop a QC plan**

When a commercial test system is used for antimicrobial susceptibility testing, refer to the manufacturer's instructions for QC **strains** and QC ranges.

General Comments

(1) Refer to Table 1J for antimicrobial agents that should be considered for testing and reporting by microbiology laboratories.

(2) For isolates for which the antimicrobial agent MICs fall within the intermediate category, maximum dosages, along with proper ancillary therapy, should be used to achieve the best possible levels of drug in abscesses and/or poorly perfused tissues. If this approach is taken, organisms for which the antimicrobial agent MICs fall within the susceptible range are generally amenable to therapy. Organisms for which the antimicrobial agent MICs are in the intermediate range may respond, but in such cases, efficacy as measured by patient clinical response should be carefully monitored. Ancillary therapy, such as drainage procedures and debridement, are of great importance for proper management of anaerobic infections.

(3) Refer to CLSI M11[1] for examples of reading end points.

(4) MIC values using either Brucella blood agar or Wilkins Chalgren agar (former reference medium) are considered equivalent.

(5) Broth microdilution is recommended only for testing *Bacteroides* spp. and *Parabacteroides* spp. MIC values for agar or broth microdilution are considered equivalent for those species.

Table 2J. Anaerobes (Continued)

(6) Until additional studies are performed to validate broth microdilution for testing other organisms, it should be used only for testing members of *Bacteroides* spp. and *Parabacteroides* spp.

NOTE: Information in boldface type is new or modified since the previous edition.

Antimicrobial Agent	Interpretive Categories and MIC Breakpoints, µg/mL			Comments
	S	I	R	
PENICILLINS				
Ampicillin Penicillin	≤ 0.5 ≤ 0.5	1 1	≥ 2 ≥ 2	**(7)** Ampicillin and penicillin are recommended for primary testing and reporting for gram-positive organisms (Tier 1; see Table 1J) because most of them are β-lactamase negative, but not for gram-negative organisms because many are β-lactamase positive (Tier 4; see Table 1J). **(8)** *Bacteroides* spp. are intrinsically resistant to penicillin and ampicillin. *Parabacteroides* spp. are presumed to be resistant to penicillin and ampicillin. Other gram-negative and gram-positive anaerobes may be screened for β-lactamase activity with a chromogenic cephalosporin; if β-lactamase positive, report as resistant to penicillin, ampicillin, and amoxicillin. Be aware that β-lactamase–negative isolates may be resistant to β-lactams by other mechanisms. Because higher blood levels are achievable with these antimicrobial agents, infection with non–β-lactamase-producing organisms with higher MICs (2–4 µg/mL) with adequate dosage regimen might be treatable. **(9)** Results of ampicillin testing can be used to predict results for amoxicillin.
β-LACTAM COMBINATION AGENTS				
(10) Organisms that test susceptible to the β-lactam agent alone are also considered susceptible to the β-lactam combination agent. However, organisms that test susceptible to the β-lactam combination agent cannot be assumed to be susceptible to the β-lactam agent alone. Similarly, organisms that test intermediate or resistant to the β-lactam agent alone may be susceptible to the β-lactam combination agent.				
Amoxicillin-clavulanate Ampicillin-sulbactam Piperacillin-tazobactam	≤ 4/2 ≤ 8/4 ≤ 16/4	8/4 16/8 32/4–64/4	≥ 16/8 ≥ 32/16 ≥ 128/4	
Imipenem-relebactam	≤ 4/4	8/4	≥ 16/4	
Ticarcillin-clavulanate*	≤ 32/2	64/2	≥ 128/2	
CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.)				
Cefotetan Cefoxitin	≤ 16 ≤ 16	32 32	≥ 64 ≥ 64	

Table 2J. Anaerobes (Continued)

Antimicrobial Agent	Interpretive Categories and MIC Breakpoints, µg/mL			Comments
	S	I	R	
CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.) (Continued)				
Ceftizoxime*	≤ 32	64	≥ 128	
Ceftriaxone	≤ 16	32	≥ 64	
Cefmetazole*	≤ 16	32	≥ 64	
Cefoperazone*	≤ 16	32	≥ 64	
Cefotaxime*	≤ 16	32	≥ 64	
CARBAPENEMS				
Doripenem*	≤ 2	4	≥ 8	
Ertapenem	≤ 4	8	≥ 16	
Imipenem	≤ 4	8	≥ 16	
Meropenem	≤ 4	8	≥ 16	
TETRACYCLINES				
Tetracycline	≤ 4	8	≥ 16	
FLUOROQUINOLONES				
Moxifloxacin	≤ 2	4	≥ 8	
LINCOSAMIDES				
Clindamycin	≤ 2	4	≥ 8	
PHENICOLS				
Chloramphenicol*	≤ 8	16	≥ 32	
NITROIMIDAZOLES				
Metronidazole	≤ 8	16	≥ 32	**(11)** Many non–spore-forming, gram-positive anaerobic rods are resistant to metronidazole.

Abbreviations: CFU, colony-forming unit(s); I, intermediate; LHB, lysed horse blood; MIC, minimal inhibitory concentration; QC, quality control; R, resistant; S, susceptible.
Symbol: *, designation for "Other" agents not included in Tables 1 but have established clinical breakpoints.

Reference for Table 2J

[1] CLSI. *Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria.* 9th ed. CLSI standard M11. Clinical and Laboratory Standards Institute; 2018.

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Introduction to Table 2 Dosages. Antimicrobial Agent Dosage Regimens Used to Establish Susceptible or Susceptible-Dose Dependent Breakpoints

The evolving science of pharmacokinetics/pharmacodynamics has become increasingly important in recent years in determining minimal inhibitory concentration breakpoints. CLSI susceptible or susceptible-dose dependent breakpoints added or revised since 2010 have been based on a specific dosage regimen(s); these dosage regimens are listed in the table below. Proper application of the breakpoints necessitates drug exposure at the site of infection that corresponds to or exceeds the expected systemic drug exposure at the dose listed in adult patients with normal renal function. This information should be shared with pharmacists, infectious diseases staff, and others making dosing recommendations for the institution.

CLSI guidance for establishing or revising breakpoints is available in CLSI M23.[1] Rationale documents that provide the scientific reasoning behind the subcommittee's decisions for some breakpoints, along with documentation of the standardized data and methods used to determine breakpoints, can be found on the CLSI website.[2]

NOTE 1: If both a susceptible and a susceptible-dose dependent dosage regimen were used, they are designated by "S" or "SDD" preceding the dosage regimen. Otherwise, it should be assumed that the dosage regimen applies to the susceptible breakpoint.

NOTE 2: Unless otherwise noted, refer to the approved prescribing information for the infusion duration used to set breakpoints for IV antibiotics (eg, 0.5 hours for most β-lactams, 1–1.5 hours for fluoroquinolones).

NOTE 3: Dosage regimens also include the frequency of administration designated by the abbreviation "q." For example, the amikacin susceptible breakpoint for Enterobacterales was based on a dosage regimen of 15 mg/kg IV q 24 h, which corresponds to 15 mg/kg IV administered every 24 hours.

NOTE 4: Information in boldface type is new or modified since the previous edition.

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Table 2 Dosages. Antimicrobial Agent Dosage Regimens Used to Establish Susceptible or Susceptible-Dose Dependent Breakpoints

Antimicrobial Agent	Dosage Regimen Used to Establish S or SDD Breakpoint
Table 2A-1. Enterobacterales (excluding *Salmonella/Shigella*)	
Amikacin	15 mg/kg IV q 24 h
Ampicillin (ampicillin test results predict results for amoxicillin)	Ampicillin: 2 g IV q 4–6 h or Amoxicillin: 1–2 g IV q 6 h
Ampicillin (ampicillin test results predict results for amoxicillin; *Escherichia coli* and *Proteus mirabilis* for uncomplicated UTIs only)	Ampicillin: 500 mg PO q 6 h or Amoxicillin: 250 mg PO q 8 h or 500 mg PO q 12 h
Amoxicillin-clavulanate (oral amoxicillin-clavulanate for uncomplicated UTIs or when completing therapy for systemic infection only)	1.2 g (1 g amoxicillin + 0.2 g clavulanate) IV q 6 h 500/125 mg PO q 8 h or 875/125 mg PO q 12 h
Ampicillin-sulbactam	3 g IV (2 g ampicillin + 1 g sulbactam) q 6 h
Aztreonam	1 g IV q 8 h
Cefazolin (*E. coli, Klebsiella pneumoniae,* and *P. mirabilis* for infections other than uncomplicated UTIs only)	2 g IV q 8 h
Cefazolin (*E. coli, K. pneumoniae,* and *P. mirabilis* for uncomplicated UTIs only)	1 g IV q 12 h
Ceftaroline	600 mg IV q 12 h
Cefepime	S: 1 g IV q 8 h or 2 g IV q 12 h SDD: 2 g IV q 8 h over 3 h
Cefiderocol	2 g IV q 8 h over 3 h
Cefotaxime	1 g IV q 8 h
Cefoxitin	8 g IV per day (eg, 2 g IV q 6 h)
Ceftriaxone	1 g IV q 24 h
Cefuroxime	1.5 g IV q 8 h
Ceftazidime	1 g IV q 8 h
Ceftazidime-avibactam	2.5 g (2 g ceftazidime + 0.5 g avibactam) IV q 8 h over 2 h
Ceftizoxime	1 g IV q 12 h
Ceftolozane-tazobactam	3 g (2 g ceftolozane + 1 g tazobactam) IV q 8 h (pneumonia) 1.5 g (1 g ceftolozane + 0.5 g tazobactam) IV q 8 h (other indications)
Ciprofloxacin	400 mg IV or 500 mg PO q 12 h

Table 2 Dosages. Antimicrobial Agent Dosage Regimens Used to Establish Susceptible or Susceptible-Dose Dependent Breakpoints (Continued)

Antimicrobial Agent	Dosage Regimen Used to Establish S or SDD Breakpoint
Table 2A-1. Enterobacterales (excluding *Salmonella/Shigella*) (Continued)	
Colistin or polymyxin B	Corresponds to intermediate breakpoint. See international consensus guidelines[3] for dosage recommendations.
Doripenem	500 mg IV q 8 h
Ertapenem	1 g IV q 24 h
Gentamicin	7 mg/kg IV q 24 h
Imipenem	500 mg IV q 6 h or 1 g IV q 8 h
Imipenem-relebactam (excluding family Morganellaceae)	1.25 g (0.5 g imipenem + 0.5 g cilastatin + 0.25 g relebactam) IV q 6 h
Levofloxacin	750 mg IV/PO q 24 h
Meropenem	500 mg IV q 6 h or 1 g IV q 8 h
Meropenem-vaborbactam	4 g (2 g meropenem + 2 g vaborbactam) IV q 8 h over 3 h
Piperacillin-tazobactam	S: 3.375–4.5 g IV q 6 h SDD: 4.5 g IV q 6 h over 3 h or 4.5 g IV q 8 h over 4 h
Plazomicin (excluding family Morganellaceae)	15 mg/kg IV q 24 h
Tobramycin	7 mg/kg IV q 24 h
Table 2A-2. *Salmonella* and *Shigella* spp.	
Ampicillin (ampicillin test results predict results for amoxicillin)	Ampicillin: 2 g IV q 4–6 h or Amoxicillin: 1–2 g IV q 6 h
Ampicillin (ampicillin test results predict results for amoxicillin)	Ampicillin: 500 mg PO q 6 h or Amoxicillin: 250 mg PO q 8 h or 500 mg PO q 12 h
Azithromycin (*S. enterica* ser. Typhi and *Shigella* spp.)	500 mg IV/PO q 24 h
Cefotaxime	1 g IV q 8 h
Ceftriaxone	1 g IV q 24 h
Ciprofloxacin	400 mg IV or 500 mg PO q 12 h
Ertapenem	1 g IV q 24 h
Imipenem	500 mg IV q 6 h or 1 g IV q 8 h
Levofloxacin	750 mg IV/PO q 24 h
Meropenem	500 mg IV q 6 h or 1 g IV q 8 h

Table 2 Dosages. Antimicrobial Agent Dosage Regimens Used to Establish Susceptible or Susceptible-Dose Dependent Breakpoints (Continued)

Antimicrobial Agent	Dosage Regimen Used to Establish S or SDD Breakpoint
Table 2B-1. *Pseudomonas aeruginosa*	
Amikacin	15 mg/kg IV q 24 h
Aztreonam	1 g IV q 6 h or 2 g IV q 8 h
Cefepime	**2 g IV q 8 h over 3 h**
Cefiderocol	2 g IV q 8 h over 3 h
Ceftazidime	1 g IV q 6 h or 2 g IV q 8 h
Ceftazidime-avibactam	2.5 g (2 g ceftazidime + 0.5 g avibactam) IV q 8 h over 2 h
Ceftolozane-tazobactam	3 g (2 g ceftolozane + 1 g tazobactam) IV q 8 h (pneumonia) 1.5 g (1 g ceftolozane + 0.5 g tazobactam) IV q 8 h (other indications)
Ciprofloxacin	400 mg IV q 8 h
Colistin or polymyxin B	Corresponds to intermediate breakpoint. See international consensus guidelines[3] for dosage recommendations.
Doripenem	500 mg IV q 8 h
Imipenem	500 mg IV q 6 h or 1 g IV q 8 h
Imipenem-relebactam	1.25 g (0.5 g imipenem + 0.5 g cilastatin + 0.25 g relebactam) IV q 6 h
Levofloxacin	750 mg IV/PO q 24 h
Meropenem	500 mg IV q 6 h or 1 g IV q 8 h
Piperacillin	4 g IV q 6 h over 0.5 h or 3 h
Piperacillin-tazobactam	4.5 g IV q 6 h over 0.5 h or 3 h
Ticarcillin-clavulanate	3 g IV q 6 h
Tobramycin	7 mg/kg IV q 24 h
Table 2B-2. *Acinetobacter* spp.	
Ampicillin-sulbactam	**3 g (2 g ampicillin + 1 g sulbactam) IV q 6 h over ≥ 3 h**
Cefiderocol (*A. baumannii* complex only)	2 g IV q 8 h over 3 h
Colistin or polymyxin B	Corresponds to intermediate breakpoint. See international consensus guidelines[3] for dosage recommendations.
Doripenem	500 mg IV q 8 h
Imipenem	500 mg IV q 6 h or 1 g IV q 8 h
Meropenem	500 mg IV q 6 h or 1 g IV q 8 h
Minocycline	**200 mg IV q 12 h**
Sulbactam-durlobactam	2 g (1 g sulbactam + 1 g durlobactam) IV q 6 h over 3 h

Table 2 Dosages. Antimicrobial Agent Dosage Regimens Used to Establish Susceptible or Susceptible-Dose Dependent Breakpoints (Continued)

Antimicrobial Agent	Dosage Regimen Used to Establish S or SDD Breakpoint
Table 2B-4. *Stenotrophomonas maltophilia*	
Cefiderocol	2 g IV q 8 h over 3 h
Minocycline	200 mg IV/PO q 12 h
Table 2C. *Staphylococcus* spp.	
Ceftaroline (*S. aureus* only)	S: 600 mg IV q 12 h SDD: 600 mg IV q 8 h over 2 h
Ceftriaxone (MSSA only)	2 g IV q 12 h; corresponds to oxacillin breakpoint
Dalbavancin (*S. aureus* only)	1500 mg IV once or 1000 mg IV once followed one wk later by 500 mg IV once
Lefamulin (*S. aureus* only)	150 mg IV or 600 mg PO q 12 h
Oritavancin (*S. aureus* only)	1200 mg IV once
Tedizolid (*S. aureus* only)	200 mg IV/PO q 24 h
Telavancin (*S. aureus* only)	10 mg/kg IV q 24 h
Table 2D. *Enterococcus* spp.	
Ampicillin (ampicillin test results predict results for amoxicillin; oral ampicillin or amoxicillin used for uncomplicated UTIs only)	Ampicillin: 2 g IV q 4–6 h or 500 mg PO q 6 h Amoxicillin: 1–2 g IV q 6 h or 250 mg PO q 8 h or 500 mg PO q 12 h
Dalbavancin (vancomycin-susceptible *E. faecalis* only)	1500 mg IV once or 1000 mg IV once followed one wk later by 500 mg IV once
Daptomycin (*E. faecium* only)	SDD: 8–12 mg/kg IV q 24 h
Daptomycin (*Enterococcus* spp. other than *E. faecium*)	6 mg/kg IV q 24 h
Oritavancin (vancomycin-susceptible *E. faecalis* only)	1200 mg IV once
Tedizolid (*E. faecalis* only)	200 mg IV/PO q 24 h
Telavancin (vancomycin-susceptible *E. faecalis* only)	10 mg/kg IV q 24 h
Table 2E. *Haemophilus influenzae* and *Haemophilus parainfluenzae*	
Amoxicillin-clavulanate	500/125 mg PO q 8 h or 875/125 mg PO q 12 h
Ampicillin (meningitis)	2 g IV q 4 h
Ampicillin-sulbactam	3 g (2 g ampicillin + 1 g sulbactam) IV q 6 h
Ceftaroline (*H. influenzae* only)	600 mg IV q 12 h
Ceftolozane-tazobactam (*H. influenzae* only)	3 g (2 g ceftolozane + 1 g tazobactam) IV q 8 h

Table 2 Dosages. Antimicrobial Agent Dosage Regimens Used to Establish Susceptible or Susceptible-Dose Dependent Breakpoints (Continued)

Antimicrobial Agent	Dosage Regimen Used to Establish S or SDD Breakpoint
Table 2E. *Haemophilus influenzae* and *Haemophilus parainfluenzae* (Continued)	
Lefamulin (*H. influenzae* only)	150 mg IV or 600 mg PO q 12 h
Table 2F. *Neisseria gonorrhoeae*	
Azithromycin	1 g IV/PO once; presumes use in an approved regimen that includes an additional agent (eg, ceftriaxone 250 mg IM once)
Table 2G. *Streptococcus pneumoniae*	
Amoxicillin (nonmeningitis)	500 mg PO q 8 h or 875 mg PO q 12 h
Amoxicillin-clavulanate (nonmeningitis)	500/125 mg PO q 8 h or 875/125 mg PO q 12 h
Ceftaroline (nonmeningitis)	600 mg IV q 12 h
Lefamulin	150 mg IV or 600 mg PO q 12 h
Penicillin (nonmeningitis)	12 million units IV per day (eg, 2 million units IV q 4 h); strains with an intermediate MIC may necessitate 18–24 million units IV per day
Penicillin (meningitis)	18 million units IV per day (eg, 3 million units IV q 4 h)
Table 2H-1. *Streptococcus* spp. β-Hemolytic Group	
Ceftaroline	600 mg IV q 12 h
Dalbavancin (*S. pyogenes*, *S. agalactiae*, and *S. dysgalactiae* only)	1500 mg IV once or 1000 mg IV once followed one wk later by 500 mg IV once
Oritavancin	1200 mg IV once
Tedizolid (*S. pyogenes* and *S. agalactiae* only)	200 mg IV/PO q 24 h
Telavancin	10 mg/kg IV q 24 h
Table 2H-2. *Streptococcus* spp. Viridans Group	
Dalbavancin (*S. anginosus* group only)	1500 mg IV once or 1000 mg IV once followed one wk later by 500 mg IV once
Oritavancin	1200 mg IV once
Tedizolid (*S. anginosus* group only)	200 mg IV/PO q 24 h
Telavancin	10 mg/kg IV q 24 h
Table 2I. *Neisseria meningitidis*	
Ampicillin	2 g IV q 4 h

Table 2 Dosages. Antimicrobial Agent Dosage Regimens Used to Establish Susceptible or Susceptible-Dose Dependent Breakpoints (Continued)

Antimicrobial Agent	Dosage Regimen Used to Establish S or SDD Breakpoint
Table 2J. Anaerobes	
Imipenem-relebactam	1.25 g (0.5 g imipenem + 0.5 g cilastatin + 0.25 g relebactam) IV q 6 h

Abbreviations: h, hour(s); IM, intramuscular; IV, intravenous; MIC, minimal inhibitory concentration; MSSA, methicillin (oxacillin) susceptible *Staphylococcus aureus*; PO, oral; q, quaque; S, susceptible; SDD, susceptible-dose dependent; UTI, urinary tract infection; wk, week(s).

References for Table 2. Dosages

[1] CLSI. *Development of* In Vitro *Susceptibility Test Methods, Breakpoints, and Quality Control Parameters*. 6th ed. CLSI guideline M23. Clinical and Laboratory Standards Institute; 2023.

[2] Clinical and Laboratory Standards Institute. Free resources from CLSI. Accessed 23 January 2024. https://clsi.org/all-free-resources/

[3] Tsuji BT, Pogue JM, Zavascki AP, et al. International consensus guidelines for the optimal use of the polymyxins: endorsed by the American College of Clinical Pharmacy (ACCP), European Society of Clinical Microbiology and Infectious Diseases (ESCMID), Infectious Diseases Society of America (IDSA), International Society for Anti-Infective Pharmacology (ISAP), Society of Critical Care Medicine (SCCM), and Society of Infectious Diseases Pharmacists (SIDP). *Pharmacotherapy.* 2019;39(1):10-39. doi:10.1002/phar.2209

Table 3A. Tests for Extended-Spectrum β-Lactamases in *Klebsiella pneumoniae, Klebsiella oxytoca, Escherichia coli,* and *Proteus mirabilis*

NOTE 1: Following evaluation of PK/PD properties, limited clinical data, and MIC distributions, revised breakpoints for cefazolin, cefotaxime, ceftazidime, ceftizoxime, ceftriaxone, and aztreonam were published in January 2010 (CLSI M100-S20) and are listed in Table 2A-1. Cefuroxime (parenteral) was also evaluated; however, no change in breakpoints was necessary with the dosage. When using the current breakpoints, routine ESBL testing is not necessary before reporting results. If ESBL testing is performed, the results may be used to guide therapeutic management or for epidemiological or infection prevention purposes.

Some phenotypic ESBL tests have known limitations that affect sensitivity (eg, false-negative results due to the coproduction of an AmpC β-lactamase) and specificity (eg, false-positive results due to hyperproduction of non-ESBL β-lactamases combined with altered permeability). Genotypic methods are limited by the targets included in the assay (eg, most FDA-cleared ESBL assays target only *bla*$_{\text{CTX-M}}$). Limitations of phenotypic and genotypic methods must be considered.

Breakpoints for drugs with limited availability in many countries (eg, moxalactam, cefonicid, cefamandole, and cefoperazone) were not evaluated. If considering use of these drugs for *E. coli, K. pneumoniae, K. oxytoca,* or *P. mirabilis,* ESBL testing should be performed. If isolates test ESBL positive, the results for moxalactam, cefonicid, cefamandole, and cefoperazone should be reported as resistant.

NOTE 2: Information in boldface type is new or modified since the previous edition.

Test	Criteria for Performance of ESBL Test		ESBL Test	
Test method	Disk diffusion	Broth microdilution	Disk diffusion	Broth microdilution
Medium	MHA	CAMHB	MHA	CAMHB
Antimicrobial concentration	For *K. pneumoniae, K. oxytoca,* and *E. coli:* Cefpodoxime 10 µg or Ceftazidime 30 µg or Aztreonam 30 µg or Cefotaxime 30 µg or Ceftriaxone 30 µg For *P. mirabilis:* Cefpodoxime 10 µg or Ceftazidime 30 µg or Cefotaxime 30 µg (Testing more than one antimicrobial agent improves the sensitivity of ESBL detection.)	For *K. pneumoniae, K. oxytoca,* and *E. coli:* Cefpodoxime 4 µg/mL or Ceftazidime 1 µg/mL or Aztreonam 1 µg/mL or Cefotaxime 1 µg/mL or Ceftriaxone 1 µg/mL For *P. mirabilis:* Cefpodoxime 1 µg/mL or Ceftazidime 1 µg/mL or Cefotaxime 1 µg/mL (Testing more than one antimicrobial agent improves the sensitivity of ESBL detection.)	Ceftazidime 30 µg Ceftazidime-clavulanate[a] 30/10 µg and Cefotaxime 30 µg Cefotaxime-clavulanate 30/10 µg (Testing necessitates using both cefotaxime and ceftazidime, alone and in combination with clavulanate.)	Ceftazidime 0.25–128 µg/mL Ceftazidime-clavulanate 0.25/4–128/4 µg/mL and Cefotaxime 0.25–64 µg/mL Cefotaxime-clavulanate 0.25/4–64/4 µg/mL (Testing necessitates using both cefotaxime and ceftazidime, alone and in combination with clavulanate.)

Table 3A. (Continued)

Test	Criteria for Performance of ESBL Test		ESBL Test	
Test method	Disk diffusion	Broth microdilution	Disk diffusion	Broth microdilution
Inoculum	Standard disk diffusion procedure	Standard broth dilution procedure	Standard disk diffusion procedure	Standard broth dilution procedure
Incubation conditions	35°C ± 2°C; ambient air	35°C ± 2°C; ambient air	35°C ± 2°C; ambient air	35°C ± 2°C; ambient air
Incubation length	16–18 h	16–20 h	16–18 h	16–20 h
Results	For *K. pneumoniae, K. oxytoca,* and *E. coli:* Cefpodoxime zone ≤ 17 mm Ceftazidime zone ≤ 22 mm Aztreonam zone ≤ 27 mm Cefotaxime zone ≤ 27 mm Ceftriaxone zone ≤ 25 mm For *P. mirabilis:* Cefpodoxime zone ≤ 22 mm Ceftazidime zone ≤ 22 mm Cefotaxime zone ≤ 27 mm Zones above may indicate ESBL production.	Growth at or above the concentrations listed may indicate ESBL production (ie, for *E. coli, K. pneumoniae,* and *K. oxytoca,* MIC ≥ 8 µg/mL for cefpodoxime or MIC ≥ 2 µg/mL for ceftazidime, aztreonam, cefotaxime, or ceftriaxone; and for *P. mirabilis,* MIC ≥ 2 µg/mL for cefpodoxime, ceftazidime, or cefotaxime).	A ≥ 5-mm increase in a zone diameter for either antimicrobial agent tested in combination with clavulanate vs the zone diameter of the agent when tested alone = ESBL (eg, ceftazidime zone = 16; ceftazidime-clavulanate zone = 21).	A ≥ 3 2-fold concentration decrease in an MIC for either antimicrobial agent tested in combination with clavulanate vs the MIC of the agent when tested alone = ESBL (eg, ceftazidime MIC = 8 µg/mL; ceftazidime-clavulanate MIC = 1 µg/mL).
Reporting			For all confirmed ESBL-producing strains: If laboratories use current cephalosporin and aztreonam breakpoints, test interpretations for these agents do not need to be changed from susceptible to resistant.	

Table 3A. (Continued)

Test	**Criteria for Performance of ESBL Test**				**ESBL Test**	
Test method	Disk diffusion		Broth microdilution		Disk diffusion	Broth microdilution
QC recommendations	When testing antimicrobial agents used for ESBL detection, *K. pneumoniae* ATCC®[b] 700603 is provided as a supplemental QC strain (eg, for training, competence assessment, or test evaluation). Either strain, *K. pneumoniae* ATCC® 700603 or *E. coli* ATCC® 25922, may then be used for routine QC (eg, daily or **per IQCP**).		When testing antimicrobial agents used for ESBL detection, *K. pneumoniae* ATCC® 700603 is provided as a supplemental QC strain (eg, for training, competence assessment, or test evaluation). Either strain, *K. pneumoniae* ATCC® 700603 or *E. coli* ATCC® 25922, may then be used for routine QC (eg, daily or **per IQCP**).		When performing the ESBL test, *K. pneumoniae* ATCC® 700603 and *E. coli* ATCC® 25922 should be used for routine QC (eg, daily or **per IQCP**).	When performing the ESBL test, *K. pneumoniae* ATCC® 700603 and *E. coli* ATCC® 25922 should be tested routinely (eg, daily or **per IQCP**).
	E. coli ATCC® 25922 (see acceptable QC ranges in Table 4A-1)		*E. coli* ATCC® 25922 = no growth (see acceptable QC ranges listed in Table 5A-1)		**Acceptable QC:** *E. coli* ATCC® 25922: ≤ 2-mm increase in zone diameter for antimicrobial agent tested in combination with clavulanate vs the zone diameter when tested alone.	**Acceptable QC:** *E. coli* ATCC® 25922: < 3 2-fold concentration decrease in MIC for antimicrobial agent tested in combination with clavulanate vs the MIC of the agent when tested alone.
	K. pneumoniae ATCC® 700603:		*K. pneumoniae* ATCC® 700603 = Growth:		*K. pneumoniae* ATCC® 700603: ≥ 5-mm increase in zone diameter of ceftazidime-clavulanate vs ceftazidime alone; ≥ 3-mm increase in zone diameter of cefotaxime-clavulanate vs cefotaxime alone.	*K. pneumoniae* ATCC® 700603: ≥ 3 2-fold concentration decrease in MIC for an antimicrobial agent tested in combination with clavulanate vs the MIC of the agent when tested alone.
	Cefpodoxime zone	9–16 mm	Cefpodoxime	MIC ≥ 8 µg/mL		
	Ceftazidime zone	10–18 mm	Ceftazidime	MIC ≥ 2 µg/mL		
	Aztreonam zone	10–16 mm	Aztreonam	MIC ≥ 2 µg/mL		
	Cefotaxime zone	17–25 mm	Cefotaxime	MIC ≥ 2 µg/mL		
	Ceftriaxone zone	16–24 mm	Ceftriaxone	MIC ≥ 2 µg/mL		

Abbreviations: ATCC®, American Type Culture Collection; CAMHB, cation-adjusted Mueller-Hinton broth; ESBL, extended-spectrum β-lactamase; FDA, US Food and Drug Administration; h, hour(s); **IQCP, individualized quality control plan;** MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; PK/PD, pharmacokinetic/pharmacodynamic; QC, quality control.

Table 3A. (Continued)

Footnotes

a. Preparation of ceftazidime-clavulanate (30 µg/10 µg) and cefotaxime-clavulanate (30 µg/10 µg) disks: Using a stock solution of clavulanate at 1000 µg/mL (either freshly prepared or taken from small aliquots that have been frozen at −70°C), add 10 µL of clavulanate to ceftazidime (30 µg) and cefotaxime (30 µg) disks. Use a micropipette to apply the 10 µL of stock solution to the ceftazidime and cefotaxime disks within one hour before they are applied to the plates, allowing about 30 minutes for the clavulanate to absorb and the disks to be dry enough for application. Use disks immediately after preparation or discard; do not store.

b. ATCC® is a registered trademark of the American Type Culture Collection.

Introduction to Tables 3B and 3C. Tests for Carbapenemases in Enterobacterales and *Pseudomonas aeruginosa*

Institutional treatment guidelines, infection prevention procedures, or epidemiological investigations may necessitate identification of carbapenemase-producing Enterobacterales and *P. aeruginosa*.[1] **Tests that detect the type of carbapenemase are recommended to inform treatment decisions in carbapenem-resistant Enterobacterales isolates.**

Carbapenemase-producing isolates of Enterobacterales usually test intermediate or resistant to one or more carbapenems using the current breakpoints as listed in Table 2A-1 (**NOTE:** Testing not susceptible to ertapenem is often the most sensitive indicator of carbapenemase production. **Depending on local epidemiology and available resources, carbapenemase testing for *Enterobacter cloacae* complex and *Klebsiella aerogenes* isolates that are only resistant to ertapenem might not be necessary. Ertapenem resistance in these species is often due to mechanisms other than carbapenemase production and carbapenemases are currently uncommon in such isolates). Carbapenemase-producing Enterobacterales** usually test resistant to one or more agents in cephalosporin subclass III (eg, cefoperazone, cefotaxime, ceftazidime, ceftizoxime, and ceftriaxone). However, some isolates that produce carbapenemases, such as **OXA-48**, SME, or IMI, often test susceptible to these cephalosporins.

	Tests Used for Carbapenemase Detection			
	Carba NP (Table 3B)	mCIM (Table 3C)	mCIM With eCIM (Table 3C)	Other (eg, molecular assays)
Organisms	Enterobacterales and *P. aeruginosa* that are not susceptible to one or more carbapenems	Enterobacterales and *P. aeruginosa* that are not susceptible to one or more carbapenems	Enterobacterales that are positive by mCIM	Enterobacterales and *P. aeruginosa* that are not susceptible to one or more carbapenems to determine the presence of a carbapenemase, or to determine carbapenemase type in isolates positive by Carba NP or mCIM
Strengths	Rapid	No special reagents or media necessary	No special reagents or media necessary	Determines type of carbapenemase in addition to absence or presence of the enzyme

Introduction to Tables 3B and 3C. (Continued)

	Tests Used for Carbapenemase Detection			
	Carba NP (Table 3B)	mCIM (Table 3C)	mCIM With eCIM (Table 3C)	Other (eg, molecular assays)
Limitations	Special reagents are needed, some of which necessitate in-house preparation (and have a short shelf life). Invalid results occur with some isolates. Certain carbapenemase types (eg, OXA-type, chromosomally encoded) are not consistently detected. **Does not determine the type of carbapenemase**	Requires overnight incubation **Does not determine the type of carbapenemase**	Requires overnight incubation **False-negative results are likely to occur for isolates coproducing a serine carbapenemase and a metallo-β-lactamase.** **Does not determine the type of serine carbapenemase or metallo-β-lactamase**	Special reagents and equipment are needed. Specific to targeted genes; false-negative result if specific carbapenemase gene present is not targeted.

Abbreviations: Carba NP, carbapenemase Nordmann-Poirel; eCIM, EDTA-modified carbapenem inactivation method; EDTA, ethylenediaminetetraacetic acid; mCIM, modified carbapenem inactivation method.

NOTE: Information in boldface type is new or modified since the previous edition.

Reference for Introduction to Tables 3B and 3C

[1] Tamma PD, Aitken SL, Bonomo RA, Mathers AJ, van Duin D, Clancy CJ. IDSA 2024 guidance on the treatment of antimicrobial resistant gram-negative infections. Accessed 15 October 2024. https://www.idsociety.org/practice-guideline/amr-guidance/

Table 3B. Carba NP Test for Suspected Carbapenemase Production in Enterobacterales and *Pseudomonas aeruginosa*[1-8]

Test	Carba NP Test
When to perform this test	For treatment (per institutional guidelines), infection prevention procedures, or epidemiological investigations. **NOTE:** No change in the interpretation of carbapenem susceptibility test results is necessary for Carba NP–positive isolates. Such testing is not currently recommended for routine use.
Test method	Colorimetric microtube assay
Test reagents and materials	• Clinical laboratory reagent water • Imipenem reference standard powder • Commercially available bacterial protein extraction reagent in Tris HCl buffer, pH 7.4 • Zinc sulfate heptahydrate • Phenol red powder • 1 N NaOH solution • 10% HCl solution • Microcentrifuge tubes 1.5 mL, clear • 1-µL inoculation loops • Containers to store prepared solutions Use reagents above to prepare the following solutions (instructions for preparation are provided below this table): • 10 mM zinc sulfate heptahydrate solution • 0.5% phenol red solution • 0.1 N NaOH solution • Carba NP Solution A • Carba NP Solution B (solution A + imipenem)
Test procedure	1. Label two microcentrifuge tubes (one “a” and one “b”) for each patient isolate, QC organism, and uninoculated reagent control. 2. Add 100 µL of bacterial protein extraction reagent to each tube. 3. For each isolate to be tested, emulsify a 1-µL loopful of bacteria from an overnight blood agar plate in both tubes “a” and “b.” Vortex each tube for 5 s. (Uninoculated reagent control tubes should contain only bacterial protein extraction reagent, no organism.) **NOTE:** Do not use growth from selective media or plates containing antibiotics or other agents that select for certain bacteria. 4. Add 100 µL of solution A to tube “a.” 5. Add 100 µL of solution B to tube “b.” 6. Vortex tubes well. 7. Incubate at 35°C ± 2°C for up to 2 h. Isolates that demonstrate positive results before 2 h can be reported as carbapenemase producers.

Table 3B. (Continued)

Test	Carba NP Test
Test interpretation	Strategy for reading (see Figure 1, below): 1. Read uninoculated reagent control tubes "a" and "b" (ie, "blanks"). • Both tubes must be red or red-orange. • If either tube is any other color, the test is invalid. 2. Read inoculated tube "a." • Tube "a" must be red or red-orange. • If tube "a" is any other color, the test is invalid. 3. Read inoculated tube "b." • Red or red-orange = negative • Light orange, dark yellow, or yellow = positive • Orange = invalid 4. Interpret results as follows:

Results for Patient and QC Tubes

Tube "a": Solution A (serves as internal control)	Tube "b": Solution B	Interpretation
Red or red-orange	Red or red-orange	Negative, no carbapenemase detected
Red or red-orange	Light orange, dark yellow, or yellow	Positive, carbapenemase producer
Red or red-orange	Orange	Invalid
Orange, light orange, dark yellow, or yellow	Any color	Invalid

NOTES:

A slight color change may be observed with the addition of imipenem to solution A. Compare patient tubes to the uninoculated reagent control tubes when interpreting questionable results.

For invalid results:

- Check reagents for QC strains and uninoculated reagent controls.

Reagent deterioration can cause invalid results. An invalid result for an uninoculated reagent control test indicates a problem with solution A and/or solution B. Check the pH of solution A. If pH is < 7.8, prepare fresh solution A and solution B.

- Repeat the test, including the uninoculated reagent controls.
- If the repeat test is invalid, perform molecular assay.

Table 3B. (Continued)

Test	Carba NP Test
Reporting	Report positive as "Carbapenemase producer." Report negative as "No carbapenemase detected."
QC recommendations	Test positive and negative QC strains and uninoculated reagent control tubes each day of testing **or as determined by IQCP**. *Klebsiella pneumoniae* ATCC®[a] BAA-1705™—carbapenemase positive *K. pneumoniae* ATCC® BAA-1706™—carbapenemase negative Results for uninoculated reagent control tubes "a" and "b" must be negative (ie, red or red-orange). Any other result invalidates all tests performed on that day with the same lot of reagents. The addition of imipenem to tube "b" might cause tube "b" to appear red-orange when tube "a" is red.

Abbreviations: ATCC®, American Type Culture Collection; Carba NP, carbapenemase Nordmann-Poirel; h, hour(s); HCl, hydrochloric acid; **IQCP, individualized quality control plan**; MIC, minimal inhibitory concentration; NaOH, sodium hydroxide; pH, negative logarithm of hydrogen ion concentration; QC, quality control; s, second(s).

Footnote

a. ATCC® is a registered trademark of the American Type Culture Collection. Per ATCC® convention, the trademark symbol is used after "BAA" in each catalog number, in conjunction with the registered ATCC® name.

NOTE 1: Test recommendations were largely derived following testing of US isolates of Enterobacterales and *P. aeruginosa* and provide for a high level of sensitivity (> 90%) and specificity (> 90%) in detecting KPC, NDM, VIM, IMP, SPM, and SME-type carbapenemases in these isolates.[7,8] The sensitivity and specificity of the test for detecting other carbapenemase production can vary. The ability of this test, as listed in the above procedure, to detect OXA-48-like producers is poor.[6,7]

NOTE 2: In CLSI studies, two KPC-positive strains with low carbapenem MICs (one *Enterobacter cloacae* susceptible by MIC to all three carbapenems and one *Escherichia coli* that was susceptible to meropenem and intermediate to imipenem and ertapenem) were not detected by this test.[7]

NOTE 3: Additional investigations of Carba NP with *Acinetobacter* spp. showed poor sensitivity (ie, 21.3% for *Acinetobacter baumannii*); therefore, the previous recommendation for use of Carba NP with *Acinetobacter* spp. was removed.[8]

Table 3B. (Continued)

Instructions for Preparing Test Components

The steps for preparing 10 mM zinc sulfate heptahydrate solution are listed below.

Step	Action	Comment
1	Weigh out 1.4 g of $ZnSO_4 \cdot 7H_2O$.	
2	Add the powder to 500 mL clinical laboratory reagent water.	
3	Mix the solution.	
4	Store the solution at room temperature.	Expiration is 1 y or not to exceed expiration of individual components.

Abbreviations: H_2O, water; $ZnSO_4$, zinc sulfate.

The steps for preparing 0.5% phenol red solution are listed below.

Step	Action	Comment
1	Weigh out 1.25 g of phenol red powder.	
2	Add the powder to 250 mL clinical laboratory reagent water.	
3	Mix the solution.	
4	Store the solution at room temperature.	Expiration is 1 y or not to exceed expiration of individual components. **NOTE:** This solution does not remain in solution. Mix well before use.

Abbreviation: y, year(s).

The steps for preparing 0.1 N sodium hydroxide solution are listed below.

Step	Action	Comment
1	Add 20 mL of 1 N NaOH to 180 mL clinical laboratory reagent water.	
2	Store the solution at room temperature.	Expiration is 1 y or not to exceed expiration of individual components.

Abbreviations: NaOH, sodium hydroxide; y, year(s).

Table 3B. (Continued)

The steps for preparing Carba NP solution A are listed below.

Step	Action	Comment
1	To a 25- to 50-mL beaker, add 2 mL of 0.5% phenol red solution to 16.6 mL clinical laboratory reagent water.	
2	Add 180 µL of 10 mM $ZnSO_4$ solution.	
3	Adjust the pH to 7.8 ± 0.1 with 0.1 N NaOH solution (or 10% HCl solution if pH is too high).	10% HCl solution can be used if the pH is too high.
4	Store the solution at 4 to 8°C in a small vial or bottle.	Protect the solution from prolonged light exposure. Expiration is 2 wk or not to exceed expiration of individual components (solution should remain red or red-orange; do not use if solution turns any other color).

Abbreviations: HCl, hydrochloric acid; NaOH, sodium hydroxide; pH, negative logarithm of hydrogen ion concentration; wk, week(s); $ZnSO_4$, zinc sulfate.

The steps for preparing Carba NP solution B (solution A + 6 mg/mL imipenem) are listed below.

Step	Action	Comment
1	Determine the amount of solution B needed, allowing 100 µL per tube for each patient, QC strain, and uninoculated reagent control.	**Example:** To test 2 patient isolates, positive and negative controls and an uninoculated reagent control, 500 µL of solution B is needed.
2	Weigh out approximately 10–20 mg of imipenem powder.	It is advisable to weigh out at least 10 mg of powder. Divide the actual weight by 6 to determine the amount (in mL) of solution A to add to the powder. **Example:** 18 mg of imipenem / 6 = 3 mL of solution A, which is sufficient for 30 tubes.
3	Store the solution at 4 to 8°C for up to 3 d.	

Abbreviations: d, day(s); QC, quality control.

Table 3B. (Continued)

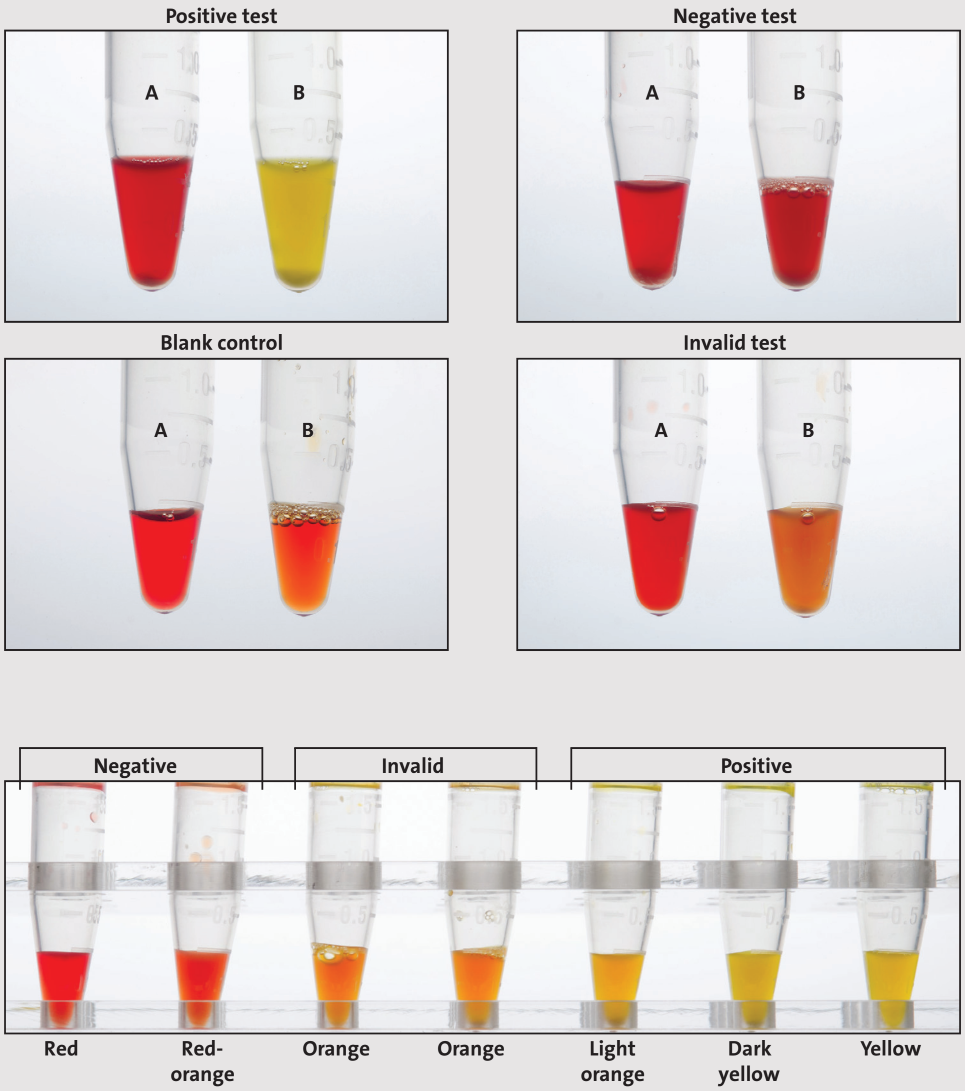
Positive test
A
B
Negative test
A
B
Blank control
A
B
Invalid test
A
B
Negative
Invalid
Positive
Red
Red-orange
Orange
Orange
Light orange
Dark yellow
Yellow

Figure 1. Interpretation of Color Reactions

Table 3B. (Continued)

References for Table 3B

[1] Nordmann P, Poirel L, Dortet L. Rapid detection of carbapenemase-producing *Enterobacteriaceae. Emerg Infect Dis.* 2012;18(9):1503-1507. doi:10.3201/eid1809.120355

[2] Dortet L, Poirel L, Nordmann P. Rapid detection of carbapenemase-producing *Pseudomonas* spp. *J Clin Microbiol.* 2012;50(11):3773-3776. doi:10.1128/JCM.01597-12

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Table 3C. Modified Carbapenem Inactivation Methods for Suspected Carbapenemase Production in Enterobacterales and *Pseudomonas aeruginosa*[1-6]

Test	mCIM Only or in Conjunction With eCIM
When to perform this test:	For treatment (per institutional guidelines), infection prevention procedures, or epidemiological investigations. **NOTE:** No change in the interpretation of carbapenem susceptibility test results is necessary for mCIM positive and/or eCIM results. mCIM with or without eCIM testing is not currently recommended for routine use. • mCIM is used for detecting carbapenemases in Enterobacterales and *P. aeruginosa* whereas eCIM is used together with mCIM to differentiate metallo-β-lactamases from serine carbapenemases in Enterobacterales. • mCIM can be performed alone; however, eCIM must be performed together with mCIM. • eCIM is valid only if mCIM is positive.
Test method	Meropenem disk inactivation
Test reagents and materials	• TSB (2 mL aliquots) • Meropenem disks (10 µg) • 1-µL and 10-µL inoculation loops • Nutrient broth (eg, Mueller-Hinton, TSB) or normal saline (3.0–5.0 mL aliquots) • MHA plates (100 mm or 150 mm) • Meropenem-susceptible indicator strain – *Escherichia coli* (ATCC®[a] 25922) • 0.5 M EDTA (only for eCIM)

Table 3C. (Continued)

Test	mCIM Only or in Conjunction With eCIM
Test procedure: mCIM	1. For each isolate to be tested, emulsify a 1-µL loopful of bacteria for Enterobacterales or 10-µL loopful of bacteria for *P. aeruginosa* from an overnight blood agar plate in 2 mL TSB. 2. Vortex for 10–15 s. 3. Add a 10-µg meropenem disk to each tube using sterile forceps or a single disk dispenser. Ensure the entire disk is immersed in the suspension. 4. Incubate at 35°C ± 2°C in ambient air for 4 h ± 15 min. 5. Just before or immediately following completion of the TSB-meropenem disk suspension incubation, prepare a 0.5 McFarland suspension (using the colony suspension method) of *E. coli* ATCC® 25922 in nutrient broth or saline. 6. Inoculate an MHA plate with *E. coli* ATCC® 25922 as for the routine disk diffusion procedure (see CLSI M02[4]) making sure the inoculum suspension preparation and MHA plate inoculation steps are each completed within 15 min. Allow the plates to dry for 3–10 min before adding the meropenem disks. 7. Remove the meropenem disk from each TSB-meropenem disk suspension using a 10-µL loop by placing the flat side of the loop against the flat edge of the disk and using surface tension to pull the disk out of the liquid. Carefully drag and press the loop along the inside edge of the tube to expel excess liquid from the disk. Continue using the loop to remove the disk from the tube and then place it on the MHA plate previously inoculated with the meropenem-susceptible *E. coli* ATCC® 25922 indicator strain. Disk capacity: 4 disks on a 100-mm MHA plate; 8 disks on a 150-mm MHA plate (see Figure 1). 8. Invert and incubate the MHA plates at 35°C ± 2°C in ambient air for 18–24 h. 9. Following incubation, measure the zones of inhibition as for the routine disk diffusion method (see CLSI M02[4]).
Test procedure: eCIM for Enterobacterales only; optional	1. For each isolate, label a second 2-mL TSB tube for the eCIM test. 2. Add 20 µL of the 0.5 M EDTA to the 2-mL TSB tube to obtain a final concentration of 5 mM EDTA. 3. Follow steps 1–9 above as for mCIM procedure. Process the mCIM and eCIM tubes in parallel. 4. Place the meropenem disks from the mCIM and eCIM tubes on the same MHA plate inoculated with the meropenem-susceptible *E. coli* ATCC® 25922 indicator strain. **NOTE:** Additional QC is needed for the eCIM test (see QC recommendations).

Table 3C. (Continued)

Test	mCIM Only or in Conjunction With eCIM
Test interpretation	For additional explanations, refer to Figures 2A, 2B, and 3A–3D, as well as the NOTEs below. mCIM • Carbapenemase positive (see Figures 2A and 2B): – Zone diameter of 6–15 mm or presence of pinpoint colonies within a 16–18-mm zone – If the test isolate produces a carbapenemase, the meropenem in the disk will be hydrolyzed and there will be no inhibition or limited growth inhibition of the meropenem-susceptible *E. coli* ATCC® 25922. • Carbapenemase negative (see Figure 2A): – Zone diameter of ≥ 19 mm (clear zone) – If the test isolate does not produce carbapenemase, the meropenem in the disk will not be hydrolyzed and will inhibit growth of the meropenem-susceptible *E. coli* ATCC® 25922. • Carbapenemase inconclusive: – Zone diameter of 16–18 mm – Zone diameter of ≥ 19 mm and the presence of pinpoint colonies within the zone – The presence or absence of a carbapenemase cannot be confirmed. eCIM – Interpret only when mCIM test is positive • Metallo-β-lactamase positive: – A ≥ 5-mm increase in zone diameter for eCIM vs zone diameter for mCIM (eg, mCIM = 6 mm; eCIM = 15 mm; zone diameter difference = 9 mm). For only the eCIM test, ignore pinpoint colonies within any zone of inhibition (see Figures 3B and 3C). – If the test isolate produces a metallo-β-lactamase, the activity of the carbapenemase will be inhibited in the presence of EDTA such that the meropenem in the disk will not be hydrolyzed as efficiently as in the tube without EDTA. The result is inhibition of the meropenem-susceptible *E. coli* and an increase in the zone diameter for the eCIM zone diameter compared with the mCIM zone diameter. • Metallo-β-lactamase **inconclusive, serine carbapenemase detected:** – A ≤ 4-mm increase in zone diameter for the eCIM vs zone diameter of mCIM (eg, mCIM = 6 mm; eCIM = 8 mm; zone diameter difference = 2 mm). For only the eCIM test, ignore pinpoint colonies within any zone of inhibition (see Figure 3D). **Isolates that coproduce a serine carbapenemase and a metallo-β-lactamase can give an inconclusive eCIM result. An alternate method should be used to rule out the presence of a metallo-β-lactamase.** – If the test isolate produces a serine carbapenemase, the activity of the carbapenemase will not be affected by the presence of EDTA and there will be no or marginal (≤ 4-mm) increase in zone diameter in the presence of EDTA compared with the mCIM zone diameter.

Table 3C. (Continued)

Test	mCIM Only or in Conjunction With eCIM
Reporting	

mCIM Only		
mCIM Result	**eCIM Result**	**Report**
Negative	Not set up	Carbapenemase not detected
Positive	Not set up	Carbapenemase detected
Inconclusive	Not set up	Testing inconclusive for the presence of carbapenemase. Call laboratory to discuss.[a]

mCIM and eCIM Combination Test		
mCIM Result	**eCIM Result**	**Report**
Negative	Do not interpret	Carbapenemase not detected
Positive	Negative	Serine carbapenemase detected; **metallo-β-lactamase inconclusive.** **Call laboratory to discuss.[b]**
Positive	Positive	Metallo-β-lactamase detected
Inconclusive	Do not interpret	Testing inconclusive for the presence of carbapenemase. Call laboratory to discuss.[a]

[a] If inconclusive **mCIM** results are obtained on repeat testing, consider performing a different phenotypic test for carbapenemase detection (ie, Carba NP) or a test for carbapenemase genes or sending isolate to a referral laboratory for further testing.

[b] If both a serine carbapenemase and a metallo-β-lactamase are coproduced by one organism, differentiation between enzymes will not be possible and false-negative eCIM results may occur, **resulting in an inconclusive interpretation for metallo-β-lactamase detection.**

Table 3C. (Continued)

<table>
<tr><th>Test</th><th>mCIM Only or in Conjunction With eCIM</th></tr>
<tr><td>NOTES</td><td>
• For mCIM inconclusive results:
– Check test isolate and <i>E. coli</i> ATCC® 25922 indicator strain for purity.
– Check meropenem disk integrity by confirming acceptable results were obtained when disks were subjected to routine disk diffusion test QC.
– Repeat the mCIM and/or eCIM for test isolate and QC strains.
• mCIM only: For some tests, pinpoint colonies of the indicator organism (<i>E. coli</i> ATCC® 25922) may be observed within the zone of inhibition. If the colonies are present within a 6–18-mm zone of inhibition, the test should be considered carbapenemase positive. If colonies are present within a ≥ 19-mm zone, the test should be considered inconclusive.
• eCIM only: Ignore pinpoint colonies within any zone of inhibition. Interpret results strictly based on the difference in zone diameters between the mCIM and eCIM tests.
• mCIM negative and eCIM positive results should not occur. If this happens, perform checks as indicated in the first bullet above. If the repeat tests are the same, consider the tests invalid.
• CLSI has currently standardized mCIM for Enterobacterales with a 1-µL loopful of bacteria and <i>P. aeruginosa</i> 10-µL loopful of bacteria only.
</td></tr>
<tr><td>QC recommendations</td><td>
Test positive and negative QC strains each day of testing (refer to Figures 2A and 2B for examples of positive and negative QC results).
<table>
<tr><th>QC Strain</th><th>Organism Characteristics</th><th>Expected Results</th></tr>
<tr><td><i>Klebsiella pneumoniae</i> ATCC® BAA-1705™</td><td>KPC positive
Serine carbapenemase producer</td><td>mCIM positive
eCIM negative</td></tr>
<tr><td><i>K. pneumoniae</i> ATCC® BAA-1706™</td><td>Carbapenemase negative</td><td>mCIM negative</td></tr>
<tr><td><i>K. pneumoniae</i> ATCC® BAA-2146™[a]</td><td>NDM positive
Metallo-β-lactamase producer</td><td>mCIM positive
eCIM positive</td></tr>
</table>
[a] eCIM positive control; to be set up only when the eCIM test is performed.
In addition, perform QC of meropenem disks and test media daily or per IQCP following the routine disk diffusion QC procedure, and handle disks as described in CLSI M02.[4] Alternatively, perform QC of meropenem disks with each run by removing a disk from the cartridge of disks used for the run and placing it on the MHA plate inoculated with <i>E. coli</i> ATCC® 25922; incubate as above.
</td></tr>
</table>

Abbreviations: ATCC®, American Type Culture Collection; Carba NP, carbapenemase Nordmann-Poirel; eCIM, EDTA-modified carbapenem inactivation method; EDTA, ethylenediaminetetraacetic acid; h, hour(s); **IQCP, individualized quality control plan;** mCIM, modified carbapenem inactivation method; MHA, Mueller-Hinton agar; min, minute(s); QC, quality control; s, second(s); TSB, trypticase soy broth.

Table 3C. (Continued)

Footnote

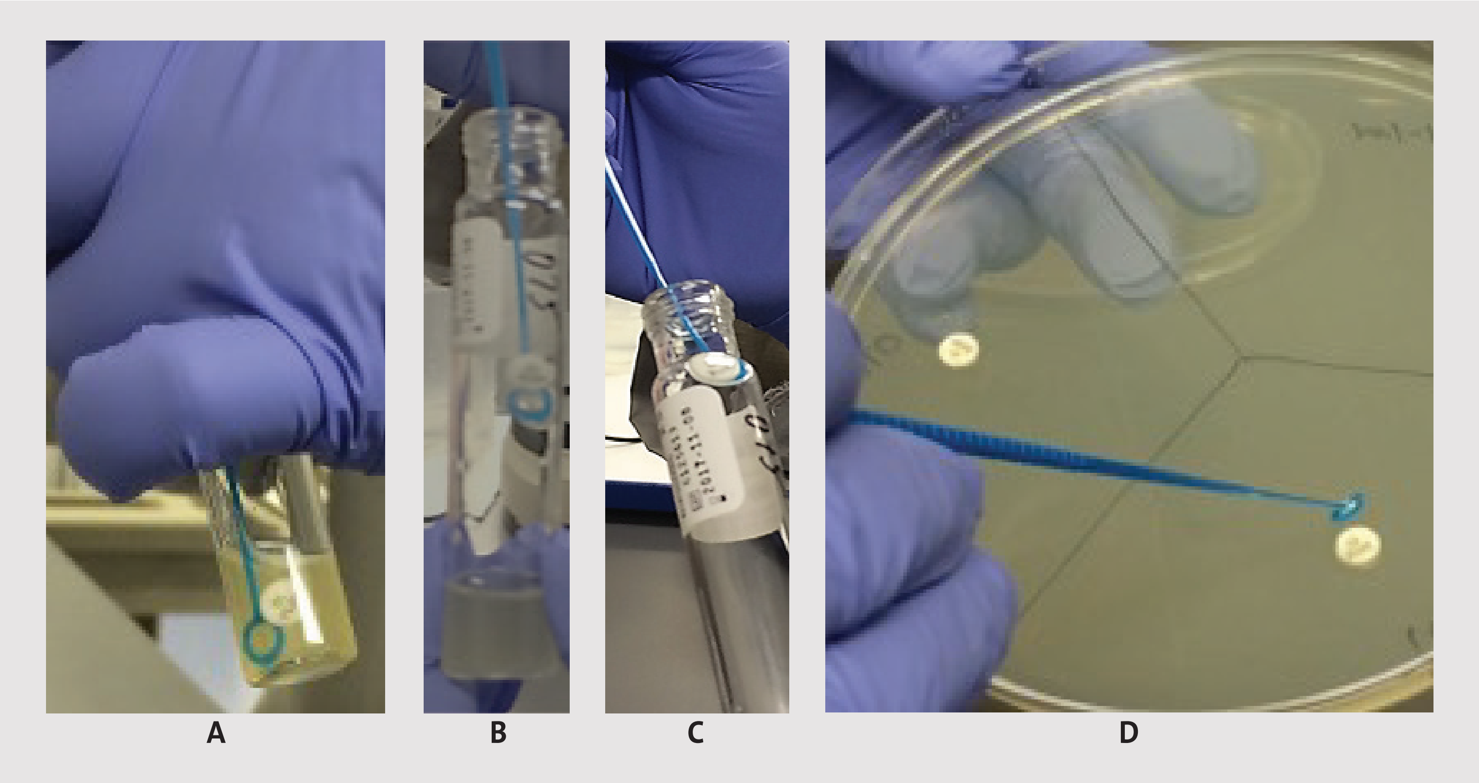
a. ATCC® is a registered trademark of the American Type Culture Collection. Per ATCC® convention, the trademark symbol is used after "BAA" in each catalog number, in conjunction with the registered ATCC® name.

NOTE 1: mCIM: This method demonstrated a sensitivity > 99% and specificity > 99% for detection of KPC, NDM, VIM, IMP, IMI, SPM, SME and OXA-type carbapenemases among Enterobacterales isolates investigated by CLSI.[3] In CLSI studies, one OXA-232–producing *K. pneumoniae* isolate was negative by this assay at 4 of 9 validation sites. This method demonstrated a sensitivity > 97% and specificity 100% for detection of KPC, NDM, VIM, IMP, IMI, SPM and OXA-type carbapenemases among *P. aeruginosa* isolates investigated by CLSI.[5] Performance for other carbapenemases or for testing isolates of non-Enterobacterales other than *P. aeruginosa* has not been established. Investigations of mCIM with *Acinetobacter* spp. showed poor specificity and poor reproducibility between laboratories, and performing mCIM with *Acinetobacter* spp. is not endorsed by CLSI.[5]

NOTE 2: eCIM: This method demonstrated a sensitivity > 95% and specificity > 92% for differentiation of metallo-β-lactamases (NDM, VIM, and IMP) from serine carbapenemases (KPC, OXA, and SME) among Enterobacterales isolates investigated by CLSI.[6] In CLSI studies, one *K. pneumoniae* coproducing NDM and OXA-181 yielded a false-negative result at 3 of 4 validation sites. **Additional studies have demonstrated poor sensitivity for detection of metallo-β-lactamases (NDM, VIM, and IMP) in isolates coproducing a serine β-lactamase (KPC or OXA-48); therefore, if eCIM is not positive, results must be considered inconclusive for metallo-β-lactamase detection.**

NOTE 3: Information in boldface type is new or modified since the previous edition.

Table 3C. (Continued)

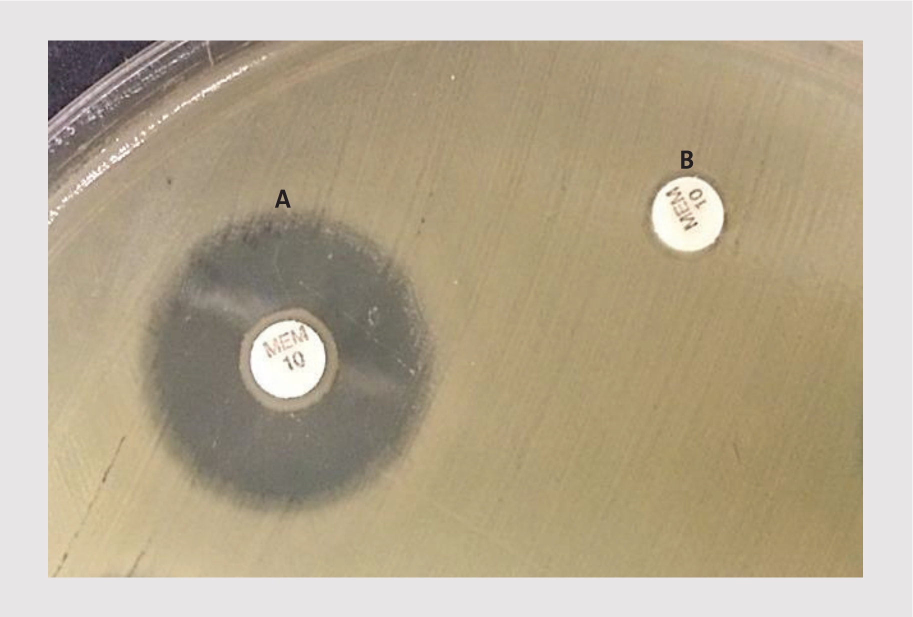


Abbreviations: ATCC®, American Type Culture Collection; mCIM, modified carbapenem inactivation method; MHA, Mueller-Hinton agar.

Figure 1. Procedure for Placing Meropenem Disks for the mCIM.

Remove the meropenem disk with a 10-µL loop (A) and drag the loop against the inside edge of the tube to expel any excess liquid (B). Use the same loop to remove the disk from the tube (C) and place it on the MHA plate (D) previously inoculated with the meropenem-susceptible *E. coli* (ATCC® 25922) indicator strain.

Table 3C. (Continued)

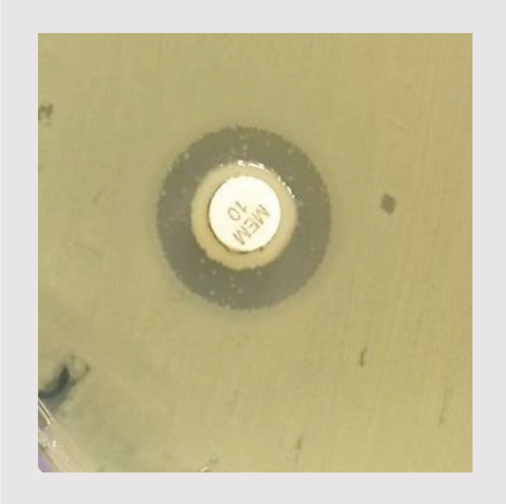


Abbreviations: ATCC®, American Type Culture Collection; mCIM, modified carbapenem inactivation method; QC, quality control; TSB, trypticase soy broth.

Figure 2A. mCIM Results for QC Strains: Negative Control *K. pneumoniae* ATCC® BAA-1706™ (A) and Positive Control *K. pneumoniae* ATCC® BAA-1705™ (B).

NOTE: A narrow ring of growth around the meropenem disk as seen with the negative control (A) results from carryover of the test organism in the TSB and should be ignored.

Table 3C. (Continued)



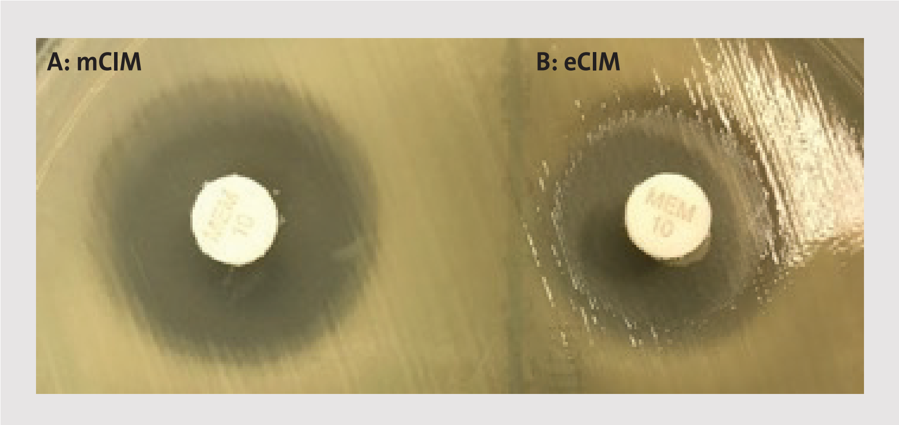
Abbreviations: mCIM, modified carbapenem inactivation method; TSB, trypticase soy broth.

Figure 2B. mCIM Test Interpretation.

- Result: positive mCIM
- Report: carbapenemase detected

NOTE: A narrow ring of growth around the meropenem disk results from carryover of the test organism in the TSB and should be ignored.

Table 3C. (Continued)



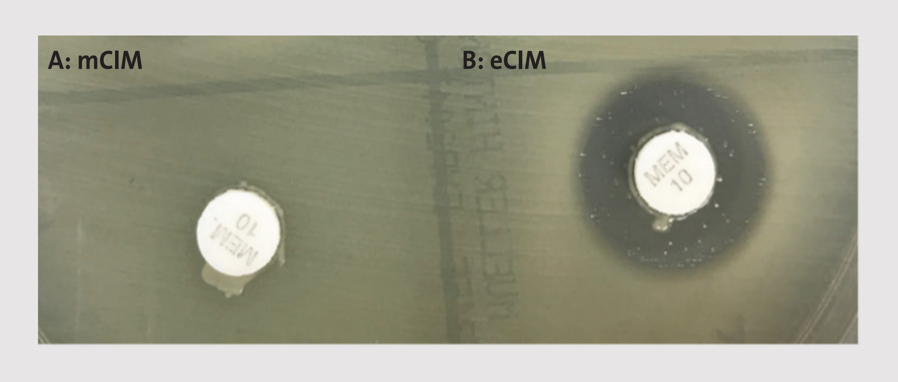
Abbreviations: eCIM, EDTA-modified carbapenem inactivation method; EDTA, ethylenediaminetetraacetic acid; mCIM, modified carbapenem inactivation method.

Figure 3A. mCIM and eCIM Test Interpretation: Negative mCIM.

An mCIM negative result (zone diameter = 20 mm) (A) and an eCIM invalid result (B). Do not interpret the eCIM result when the mCIM is negative as the isolate is negative for carbapenemase production.

- Result: negative for carbapenemase production
- Report: carbapenemase not detected

Table 3C. (Continued)



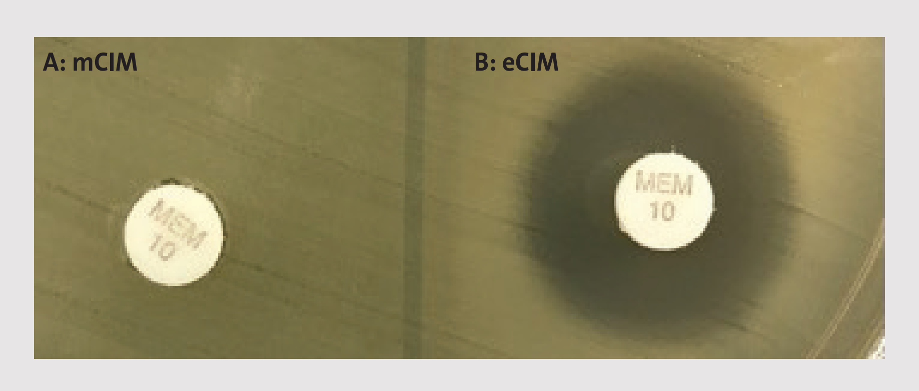
Abbreviations: eCIM, EDTA-modified carbapenem inactivation method; EDTA, ethylenediaminetetraacetic acid; mCIM, modified carbapenem inactivation method.

Figure 3B. mCIM and eCIM Test Interpretation: Positive mCIM and eCIM.

An mCIM positive result (zone diameter of 6 mm) (A) and an eCIM positive result (zone diameter = 15 mm with pinpoint colonies throughout the zone of inhibition) (B). **NOTE:** The pinpoint colonies throughout the zone of inhibition are ignored when measuring the zone for the eCIM test. A ≥ 5-mm increase in zone diameter for eCIM vs zone diameter for mCIM (15 mm – 6 mm = 9 mm) demonstrates the inhibition of the metallo-β-lactamase in the presence of EDTA.

- Result: positive mCIM and eCIM
- Report: metallo-β-lactamase detected

Table 3C. (Continued)



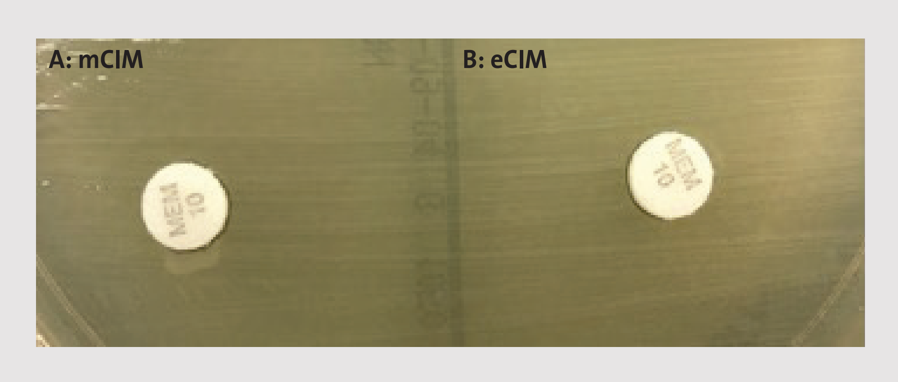
Abbreviations: eCIM, EDTA-modified carbapenem inactivation method; EDTA, ethylenediaminetetraacetic acid; mCIM, modified carbapenem inactivation method.

Figure 3C. mCIM and eCIM Test Interpretation: Positive mCIM and eCIM.

An mCIM positive result (zone diameter = 6 mm) (A) and an eCIM positive result (zone diameter = 19 mm) (B). A ≥ 5-mm increase in zone diameter for eCIM vs diameter for mCIM zone (19 mm – 6 mm = 13 mm) demonstrates the inhibition of the metallo-β-lactamase in the presence of EDTA.

- Result: positive mCIM and eCIM
- Report: metallo-β-lactamase detected

Table 3C. (Continued)



Abbreviations: eCIM, EDTA-modified carbapenem inactivation method; EDTA, ethylenediaminetetraacetic acid; mCIM, modified carbapenem inactivation method.

Figure 3D. mCIM and eCIM Test Interpretation: Positive mCIM and Negative eCIM.

An mCIM positive result (zone diameter = 6 mm) (A) and an eCIM negative result (zone diameter = 6 mm) (B). Serine carbapenemases are not inhibited by EDTA and demonstrate a ≤ 4-mm increase in zone diameter for eCIM vs zone diameter for mCIM.

- Result: positive mCIM and negative eCIM
- Report: serine carbapenemase detected

Table 3C. (Continued)

References for Table 3C

[1] Tijet N, Patel SN, Melano RG. Detection of carbapenemase activity in Enterobacteriaceae: comparison of the carbapenem inactivation method versus the Carba NP test. *J Antimicrob Chemother.* 2016;71(1):274-276. doi:10.1093/jac/dkv283

[2] van der Zwaluw K, de Haan A, Pluister GN, Bootsma HJ, de Neeling AJ, Schouls LM. The carbapenem inactivation method (CIM), a simple and low-cost alternative for the Carba NP test to assess phenotypic carbapenemase activity in gram-negative rods. *PLoS One.* 2015;10(3):e0123690. doi:10.1371/journal.pone.0123690

[3] Pierce VM, Simner PJ, Lonsway DR, et al. Modified carbapenem inactivation method for phenotypic detection of carbapenemase production among Enterobacteriaceae. *J Clin Microbiol.* 2017;55(8):2321-2333. doi:10.1128/JCM.00193-17

[4] CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests.* 14th ed. CLSI standard M02. Clinical and Laboratory Standards Institute; 2024.

[5] Simner PJ, Johnson JK, Brasso WB, et al. Multicenter evaluation of the modified carbapenem inactivation method and the Carba NP for detection of carbapenemase-producing *Pseudomonas aeruginosa* and *Acinetobacter baumannii. J Clin Microbiol.* 2017;56(1):e01369-17. doi:10.1128/JCM.01369-17

[6] Sfeir MM, Hayden JA, Fauntleroy KA, et al. EDTA-modified carbapenem inactivation method: a phenotypic method for detecting metallo-β-lactamase-producing Enterobacteriaceae. *J Clin Microbiol.* 2019;57(5):e01757-18. doi:10.1128/JCM.01757-18

Table 3D. Aztreonam Plus Ceftazidime-Avibactam Broth Disk Elution Method[1]

Due to limited therapeutic options, there may be a clinical need to assess the *in vitro* activity of the combination of aztreonam and ceftazidime-avibactam to guide therapeutic management of multidrug-resistant gram-negative bacterial infections, especially those caused by MBL producers.

The aztreonam plus ceftazidime-avibactam broth disk elution method was established with limited disk and/or media manufacturers and is considered provisional until additional data are evaluated by CLSI and shown to meet CLSI M23[2] guidance.

NOTE 1: Manufacturer-related issues were observed with different combinations of antimicrobial disks and CAMHB when the aztreonam plus ceftazidime-avibactam broth disk elution method was performed. QC of the method must be performed with every new lot or shipment of reagents to ensure the accuracy of results.

NOTE 2: Information in boldface type is new or modified since the previous edition.

Test	Aztreonam Plus Ceftazidime-Avibactam Broth Disk Elution
Organism group	Enterobacterales and *Stenotrophomonas maltophilia*
When to perform this test	Testing multidrug-resistant isolates, especially MBL producers
Test method	Tube dilution using aztreonam and ceftazidime-avibactam disks as the antimicrobial source
Medium	CAMHB (5-mL tubes)
Antimicrobial concentration	30-µg aztreonam disks 30/20-µg ceftazidime-avibactam disks Final concentration: 6 µg/mL aztreonam, 6 µg/mL ceftazidime, 4 µg/mL avibactam
Inoculum	1. Using a loop or swab, pick 3–5 colonies from a fresh (18–24 h) nonselective agar plate and transfer to sterile saline (4–5 mL). 2. Adjust turbidity to equivalent of a 0.5 McFarland turbidity standard.

Table 3D. (Continued)

Test	Aztreonam Plus Ceftazidime-Avibactam Broth Disk Elution
Test procedure	1. Let the CAMHB tubes (5 mL) and antimicrobial disks warm to room temperature. 2. Label 4 tubes of CAMHB for each isolate to be tested with ATM, CZA, ATM + CZA, and GC (see Figure 1). 3. Using aseptic technique, carefully add: • 1 aztreonam disk to the tube labeled "ATM" • 1 ceftazidime-avibactam disk to the tube labeled "CZA" • 1 aztreonam AND 1 ceftazidime-avibactam disk to the tube labeled "ATM + CZA" 4. Gently vortex the tubes with the added disk(s) and let the antimicrobial agent(s) elute from the disks for at least 30 min but no longer than 60 min at room temperature. 5. Prepare the standardized inoculum. 6. Add 25-µL standardized inoculum to the GC, ATM, CZA, and ATM + CZA tubes to attain a final inoculum concentration of approximately 7.5 × 10^5 CFU/mL. 7. Using a 10-µL loop, subculture from the original inoculum tube to a blood agar plate as a purity check. 8. Cap the tubes tightly and vortex each inoculated tube on slow speed to mix. Slow speed is suggested to prevent the disks from sticking to the cap and glass surface above the meniscus of liquid. 9. Loosen the caps slightly before incubation. 10. Incubate the tubes and purity plate.
Incubation conditions	33 to 35°C; ambient air
Incubation length	16–20 h
Results	1. Examine the purity plate to ensure inoculum was pure. 2. Examine the GC tube, which must demonstrate obvious turbidity for the test to be valid. 3. Examine each of the ATM, CZA, and ATM + CZA tubes for growth or no growth. Any turbidity noted by the naked eye should be reported as "growth." For Enterobacterales and *S. maltophilia*: • No growth = susceptible • Growth = not susceptible

Table 3D. (Continued)

<table>
<tr><th>Test</th><th>Aztreonam Plus Ceftazidime-Avibactam Broth Disk Elution</th></tr>
<tr><td>Additional testing and reporting</td><td>If the growth pattern is inconsistent (eg, no growth in ATM and growth in both CZA and ATM + CZA tubes), repeat the test. An inconsistent growth pattern may occur as a result of:
• Contamination
• Improper concentrations of antimicrobial agent in the tubes
• Manufacturer-related issues relating to the combination of disks and CAMHB
• Tube inoculation error</td></tr>
<tr><td>QC recommendations – routine[a,b,c]</td><td>
<table>
<tr><th>QC Strain</th><th>Organism Characteristics</th><th>Expected Results</th></tr>
<tr><td><i>Escherichia coli</i> ATCC®[d] 25922</td><td>Susceptible to all antimicrobial agents evaluated (see Figure 1)</td><td>ATM: No growth – susceptible
CZA: No growth – susceptible
ATM + CZA: No growth – susceptible</td></tr>
<tr><td><i>Klebsiella pneumoniae</i> ATCC® BAA-1705™</td><td>Not susceptible to ATM. Susceptible to CZA and ATM + CZA (see Figure 2)</td><td>ATM: Growth – not susceptible
CZA: No growth – susceptible
ATM + CZA: No growth – susceptible</td></tr>
<tr><td><i>K. pneumoniae</i> ATCC® BAA-2146™</td><td>Not susceptible to ATM or CZA. Susceptible to ATM + CZA (see Figure 3)</td><td>ATM: Growth – not susceptible
CZA: Growth – not susceptible
ATM + CZA: No growth – susceptible</td></tr>
<tr><td><i>E. coli</i> AR Bank #0348[e]
Alternative strains: <i>E. coli</i> AR Bank #0434[e] or <i>E. coli</i> AR Bank #0450[e]</td><td>Not susceptible to any antimicrobial agents evaluated (see Figure 4)</td><td>ATM: Growth – not susceptible
CZA: Growth – not susceptible
ATM + CZA: Growth – not susceptible</td></tr>
</table>
</td></tr>
</table>

Abbreviations: AR, antimicrobial resistance; ATCC®, American Type Culture Collection; ATM, aztreonam; ATM + CZA, aztreonam plus ceftazidime-avibactam; CAMHB, cation-adjusted Mueller-Hinton broth; CFU, colony-forming unit(s); CZA, ceftazidime-avibactam; FDA, US Food and Drug Administration; GC, growth control; h, hour(s); **IQCP, individualized quality control plan;** MBL, metallo-β-lactamase; min, minute(s); QC, quality control.

Table 3D. (Continued)

Footnotes

a. QC recommendations – routine:

- Daily if the test is performed at least once a week and/or if **an IQCP justifying less frequent QC has not been developed**
- **Less frequent than daily if the test is performed at least once per week and an IQCP has been developed**

Perform QC of antimicrobial disks and test media daily or **per IQCP** following the routine disk diffusion QC procedure and handle disks as described in CLSI M02.[3]

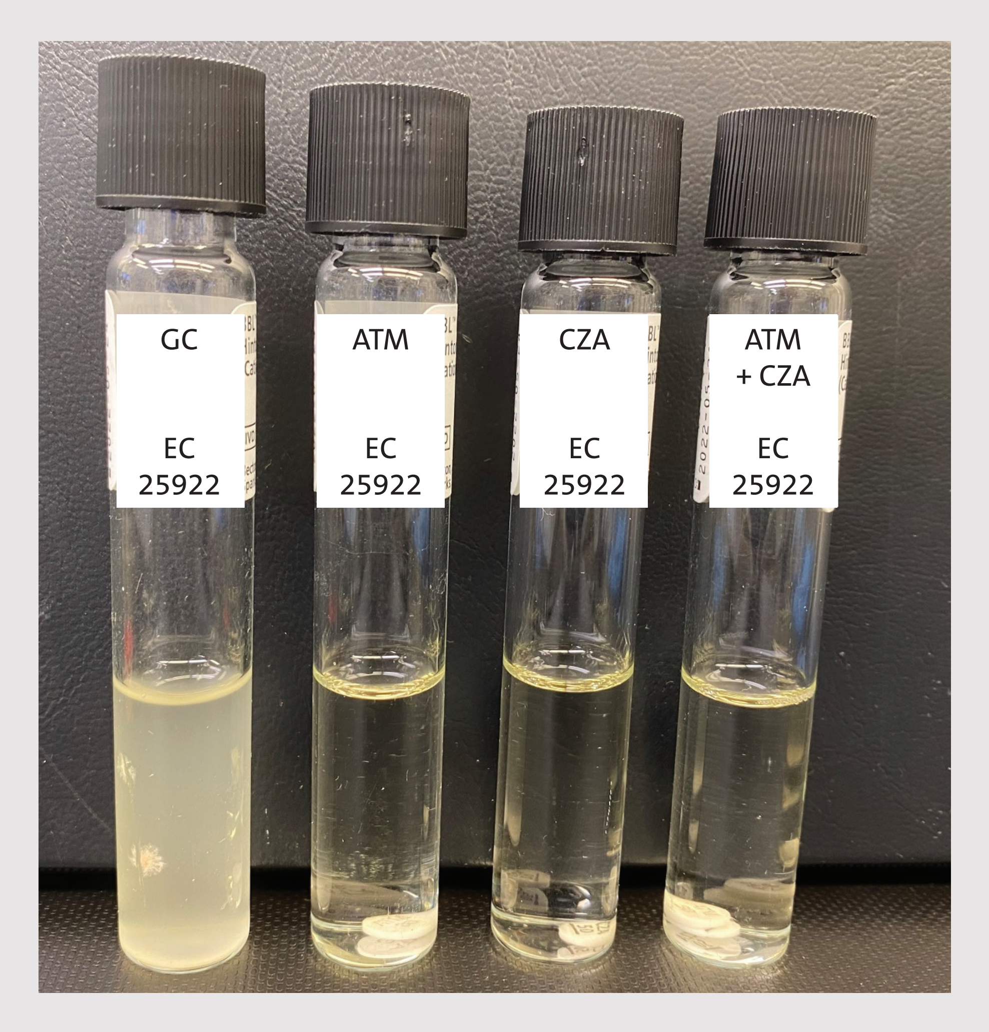
b. Manufacturer-related issues were observed with different combinations of antimicrobial disks and CAMHB when the aztreonam plus ceftazidime-avibactam broth disk elution method was performed. QC of the method must be performed with every new lot or shipment of reagents to ensure the accuracy of results. The inclusion of a not susceptible aztreonam plus ceftazidime-avibactam control is required (eg, *E. coli* AR Bank #0348).

c. The QC ranges were established with disks and media from a limited number of manufacturers and are considered provisional until additional data are evaluated by CLSI and shown to meet CLSI M23[2] guidance.

d. ATCC® is a registered trademark of the American Type Culture Collection.

e. The AR Isolate Bank (https://wwwn.cdc.gov/arisolatebank/) is a centralized repository of microbial pathogens with well-characterized resistance profiles that are assembled by the Centers for Disease Control and Prevention in collaboration with the FDA.

Table 3D. (Continued)

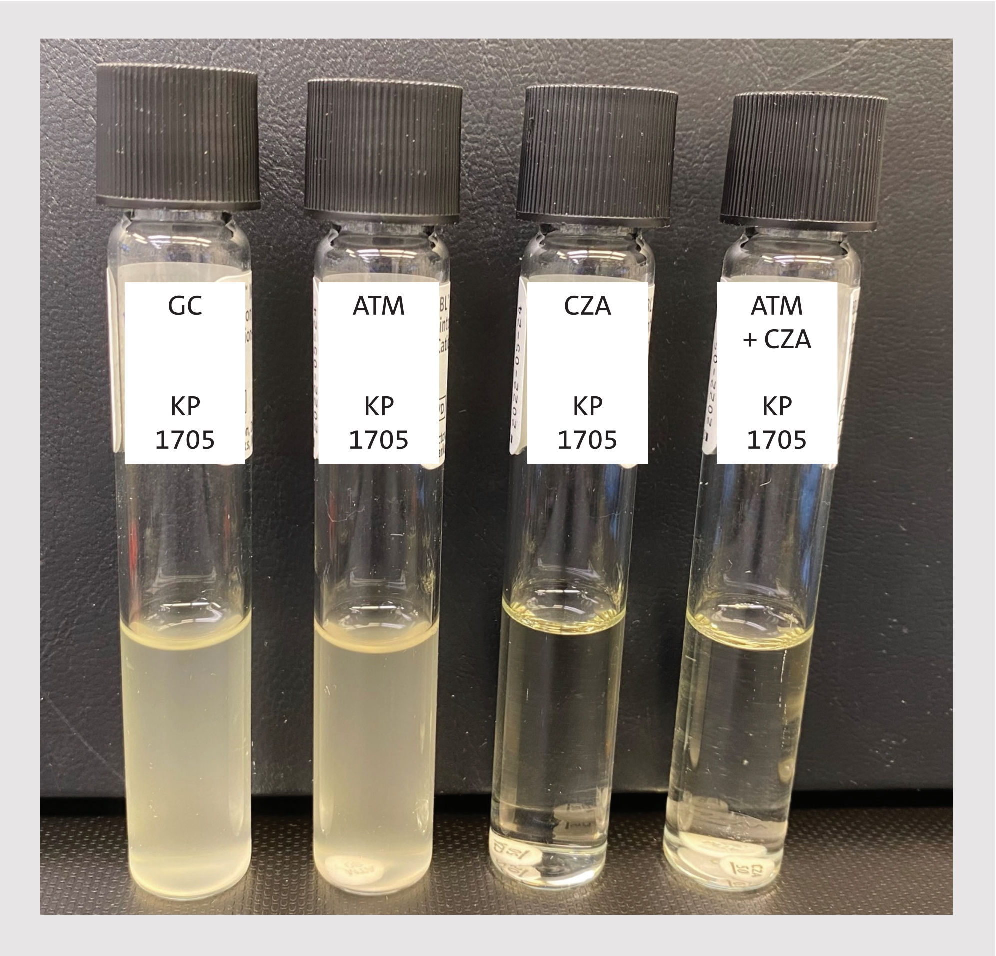


Abbreviations: ATCC®, American Type Culture Collection; ATM, aztreonam; ATM + CZA, aztreonam plus ceftazidime-avibactam; CZA, ceftazidime-avibactam; EC 25922, *Escherichia coli* ATCC® 25922; GC, growth control; QC, quality control.

Figure 1. Aztreonam Plus Ceftazidime-Avibactam Broth Disk Elution.

Results for routine QC strain EC 25922 demonstrating growth in the GC tube and no growth in the ATM, CZA, or ATM + CZA tubes. The result would be interpreted as susceptible to ATM, CZA, and ATM + CZA.

Table 3D. (Continued)

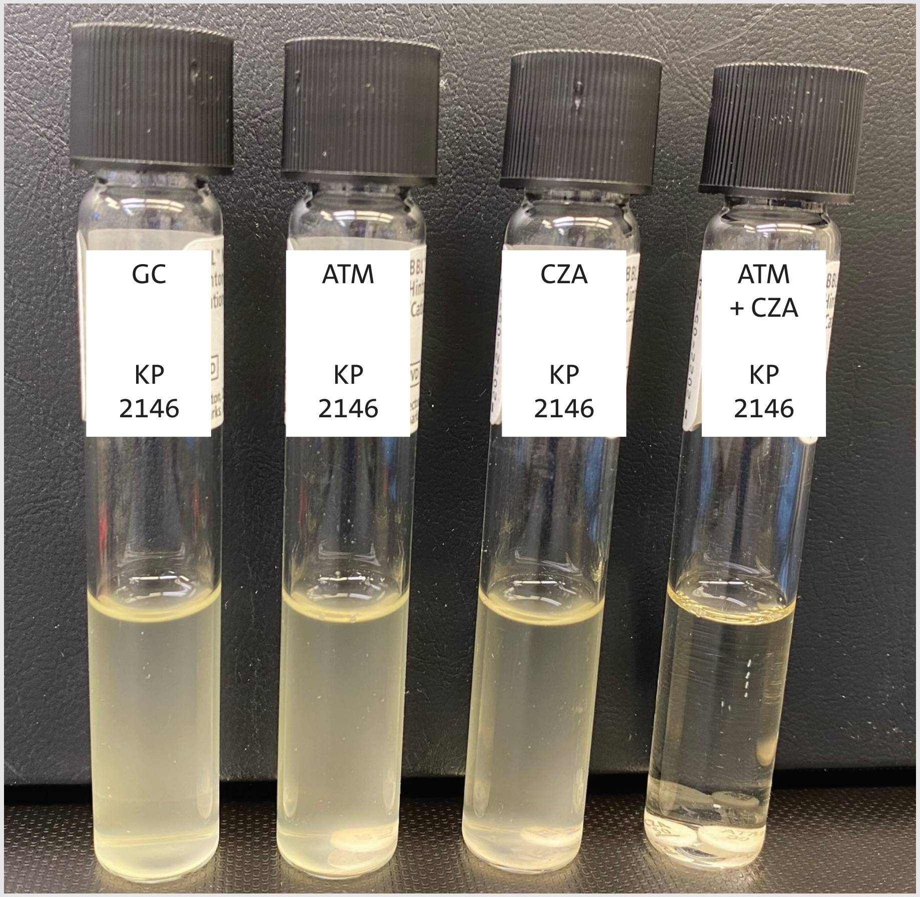


Abbreviations: ATCC®, American Type Culture Collection; ATM, aztreonam; ATM + CZA, aztreonam plus ceftazidime-avibactam; CZA, ceftazidime-avibactam; GC, growth control; KP 1705, *Klebsiella pneumoniae* ATCC® BAA-1705™; QC, quality control.

Figure 2. Aztreonam Plus Ceftazidime-Avibactam Broth Disk Elution.

Results for routine QC strain KP 1705 demonstrating growth in the GC and ATM tubes and no growth in the CZA or ATM + CZA tubes. The result would be interpreted as not susceptible to ATM, but susceptible to CZA and ATM + CZA.

Table 3D. (Continued)

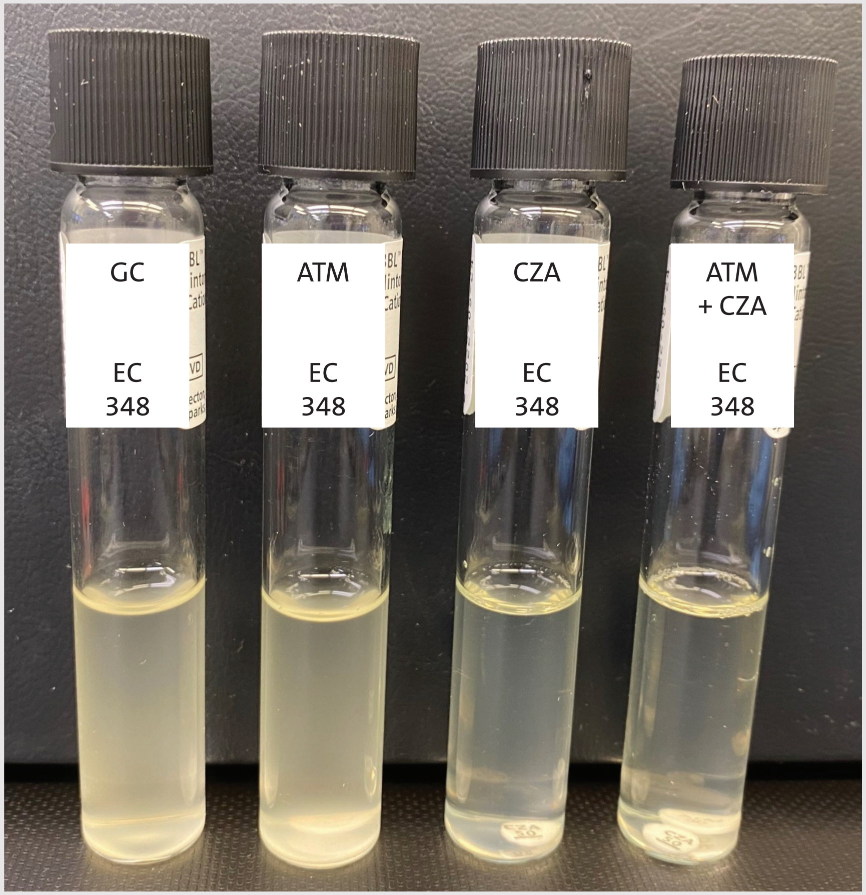


Abbreviations: ATCC®, American Type Culture Collection; ATM, aztreonam; ATM + CZA, aztreonam plus ceftazidime-avibactam; CZA, ceftazidime-avibactam; GC, growth control; KP 2146, *Klebsiella pneumoniae* ATCC® BAA-2146™; QC, quality control.

Figure 3. Aztreonam Plus Ceftazidime-Avibactam Broth Disk Elution.

Results for routine QC strain KP 2146 demonstrating growth in the GC, ATM, and CZA tubes and no growth in the ATM + CZA tube. The result would be interpreted as not susceptible to ATM or CZA, but susceptible to ATM + CZA.

Table 3D. (Continued)



Abbreviations: ATM, aztreonam; ATM + CZA, aztreonam plus ceftazidime-avibactam; CZA, ceftazidime-avibactam; EC 348, *Escherichia coli* Antimicrobial Resistance Bank #0348; GC, growth control; QC, quality control.

Figure 4. Aztreonam Plus Ceftazidime-Avibactam Broth Disk Elution.

Results for routine supplemental QC strain EC 348 demonstrating growth in the GC, ATM, CZA, and ATM + CZA tubes. The result would be interpreted as not susceptible to ATM, CZA, or ATM + CZA.

Table 3D. (Continued)

References for Table 3D

[1] Harris H, Tao L, Jacobs EB, et al. Multicenter evaluation of an MIC-based aztreonam and ceftazidime-avibactam broth disk elution test. *J Clin Microbiol.* 2023;61(5):e0164722. doi:10.1128/jcm.01647-22

[2] CLSI. *Development of* In Vitro *Susceptibility Test Methods, Breakpoints, and Quality Control Parameters.* 6th ed. CLSI guideline M23. Clinical and Laboratory Standards Institute; 2023.

[3] CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests.* 14th ed. CLSI standard M02. Clinical and Laboratory Standards Institute; 2024.

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Table 3E. Tests for Colistin Resistance for Enterobacterales and *Pseudomonas aeruginosa*

The polymyxins (colistin and polymyxin B) are antimicrobial agents of last resort for treating multidrug-resistant infections. Clinical and PK/PD data suggest that these agents have limited clinical efficacy. Alternative agents are strongly preferred. If these agents are not available, knowledge of the colistin MIC may be helpful to inform treatment decisions.

For colistin, broth microdilution, broth disk elution and agar dilution MIC methods are acceptable. Broth microdilution is the only approved method for polymyxin B. Disk diffusion and gradient diffusion methods should not be performed.

Colistin and polymyxin B are considered equivalent agents, so MICs obtained from testing colistin predict MICs to polymyxin B and vice versa. At this time, CLSI has not evaluated polymyxin B testing methods, and the procedures below should not be adapted to polymyxin B. The methods below were evaluated for *Acinetobacter* spp. by CLSI and found to yield inaccurate results.

These methods were established with limited disk and/or media manufacturers and are considered provisional until additional data are evaluated by CLSI and shown to meet CLSI M23[1] guidance.

Test	Colistin Broth Disk Elution	Colistin Agar Test
Organism group	Enterobacterales and *P. aeruginosa*	Enterobacterales and *P. aeruginosa*
Strengths	No special reagents or media necessary	Ability to test up to 10 isolates at one time
Limitations	Hands-on time and cost	Requires special media (colistin agar plate)
When to perform this test	Testing multidrug-resistant isolates for clinical or infection prevention purposes	Testing multidrug-resistant isolates for clinical or infection prevention purposes
Test method	Tube dilution using colistin disk as the colistin source	Agar dilution: slight variation of method described in CLSI M07[2] (ie, different inoculum and different approach to interpreting results)
Medium	CAMHB (10-mL tubes)	MHA (20 mL in 100-mm Petri plate)[a]
Antimicrobial concentration	10-µg colistin sulfate disks Final concentration: 0 µg/mL (GC), 1 µg/mL, 2 µg/mL, and 4 µg/mL colistin	Colistin sulfate Final concentration: 0 µg/mL (GC), 1 µg/mL, 2 µg/mL, and 4 µg/mL colistin[a]
Inoculum	1. Using a loop or swab, pick 3–5 colonies from a fresh (18–24 h) nonselective agar plate and transfer to sterile saline (4–5 mL). 2. Adjust turbidity to equivalent of a 0.5 McFarland turbidity standard.	1. Using a loop or swab, pick 3–5 colonies from a fresh (18–24 h) nonselective agar plate and transfer to sterile saline (4–5 mL). 2. Adjust turbidity to equivalent of a 0.5 McFarland turbidity standard. 3. Dilute the standardized inoculum 1:10 in saline.

Table 3E. (Continued)

Test	Colistin Broth Disk Elution	Colistin Agar Test
Test procedure	1. Let the CAMHB tubes (10 mL) and colistin disks warm to room temperature. 2. Label 4 tubes of CAMHB for each isolate to be tested with 1, 2, and 4 µg/mL and control (see Figure 1). 3. Using aseptic technique, carefully add: • 1 colistin disk to the tube labeled "1 µg/mL" • 2 colistin disks to tube labeled "2 µg/mL" • 4 colistin disks to the tube labeled "4 µg/mL" 4. Gently vortex the tubes with the added disk and let the colistin elute from the disks for at least 30 min but no longer than 60 min at room temperature. 5. Prepare the standardized inoculum. 6. Add 50 µL standardized inoculum to the control and 1-, 2-, and 4-µg/mL tubes to attain a final inoculum concentration of approximately 7.5 × 10^5 CFU/mL. 7. Using a 10-µL loop, subculture from the original inoculum tube to a blood agar plate as a purity check. 8. Cap the tubes tightly and vortex each inoculated tube on slow speed to mix. Slow speed is suggested to prevent colistin from sticking to the cap and glass surface above the meniscus of liquid. 9. Loosen the caps slightly before incubation. 10. Incubate the tubes and purity plate.	1. Divide each colistin agar plate with increasingly doubled dilutions of colistin in up to 10 parts, with a marker to test up to 10 isolates per plate. Label each part with the appropriate isolate number (see Figure 2). 2. Using a pipette or a 10-µL loop, streak 10 µL of the 1:10 dilution onto the appropriate part of each colistin agar plate. 3. Using a 10-µL loop, subculture from the original inoculum tube to a blood agar plate as a purity check. 4. Incubate the colistin agar plates and purity plate.
Incubation conditions	33 to 35°C; ambient air	33 to 35°C; ambient air
Incubation length	16–20 h	16–20 h

Table 3E. (Continued)

Test	Colistin Broth Disk Elution	Colistin Agar Test
Results	1. Examine the purity plate to ensure inoculum was pure. 2. Examine the GC tube, which must demonstrate obvious turbidity for the test to be valid. **NOTE:** Some *P. aeruginosa* isolates may grow only near the meniscus. 3. Read the MIC as the lowest concentration that completely inhibits growth of the test isolate. (See Figure 1 for examples.) For Enterobacterales and *P. aeruginosa*: • ≤ 2 µg/mL = intermediate • ≥ 4 µg/mL = resistant	1. Examine the purity plate to ensure inoculum was pure. 2. Examine the GC plate, which must demonstrate confluent growth for the test to be valid. 3. Examine the colistin plates carefully with transmitted light for colony or light film of growth. 4. Read the MIC as the lowest colistin agar plate concentration that completely inhibits growth of the test isolate (eg, even 1 colony would be considered growth). See Figure 2 for examples. For Enterobacterales and *P. aeruginosa:* • ≤ 2 µg/mL = intermediate • ≥ 4 µg/mL = resistant
Additional testing and reporting	If there is an inconsistent growth pattern (eg, no growth in 2 µg/mL but growth at 1 µg/mL and 4 µg/mL), repeat the test. An inconsistent growth pattern may occur as a result of: • Contamination at higher dilutions • Heteroresistance • Improper concentrations of antimicrobial agent in the tubes • Error inoculating the tubes	If there is an inconsistent growth pattern (eg, no growth in 2 µg/mL but growth at 1 µg/mL and 4 µg/mL), repeat the test. An inconsistent growth pattern may occur as a result of: • Contamination at higher dilutions • Heteroresistance • Improper concentrations of antimicrobial agent in the colistin agar plates • Error inoculating the plates
QC recommendations – routine[b,c]	*Escherichia coli* ATCC®[d] BAA-3170™ (formerly AR Bank #0349 *mcr-1*) (≤ 1–4 µg/mL, with a mode of 2 µg/mL)[c] and *P. aeruginosa* ATCC® 27853 (≤ 1–4 µg/mL)	*E. coli* ATCC® BAA-3170™ (formerly AR Bank #0349 *mcr-1*) (≤ 1–4 µg/mL, with a mode of 2 µg/mL)[c] and *P. aeruginosa* ATCC® 27853 (≤ 1–4 µg/mL)

Abbreviations: AR, antimicrobial resistance; ATCC®, American Type Culture Collection; CAMHB, cation-adjusted Mueller-Hinton broth; CFU, colony-forming unit(s); GC, growth control; h, hour(s); **IQCP, individualized quality control plan**; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; PK/PD, pharmacokinetic/pharmacodynamic; QC, quality control.

Table 3E. (Continued)

Footnotes

a. Refer to CLSI M07[2] for preparation of media and antimicrobial agents.

b. QC recommendations – routine

- Daily if the test is performed less than once per week and/or if **an IQCP justifying less frequent QC has not been developed**
- **Less frequent than daily if the test is performed at least once per week and an IQCP has been developed**

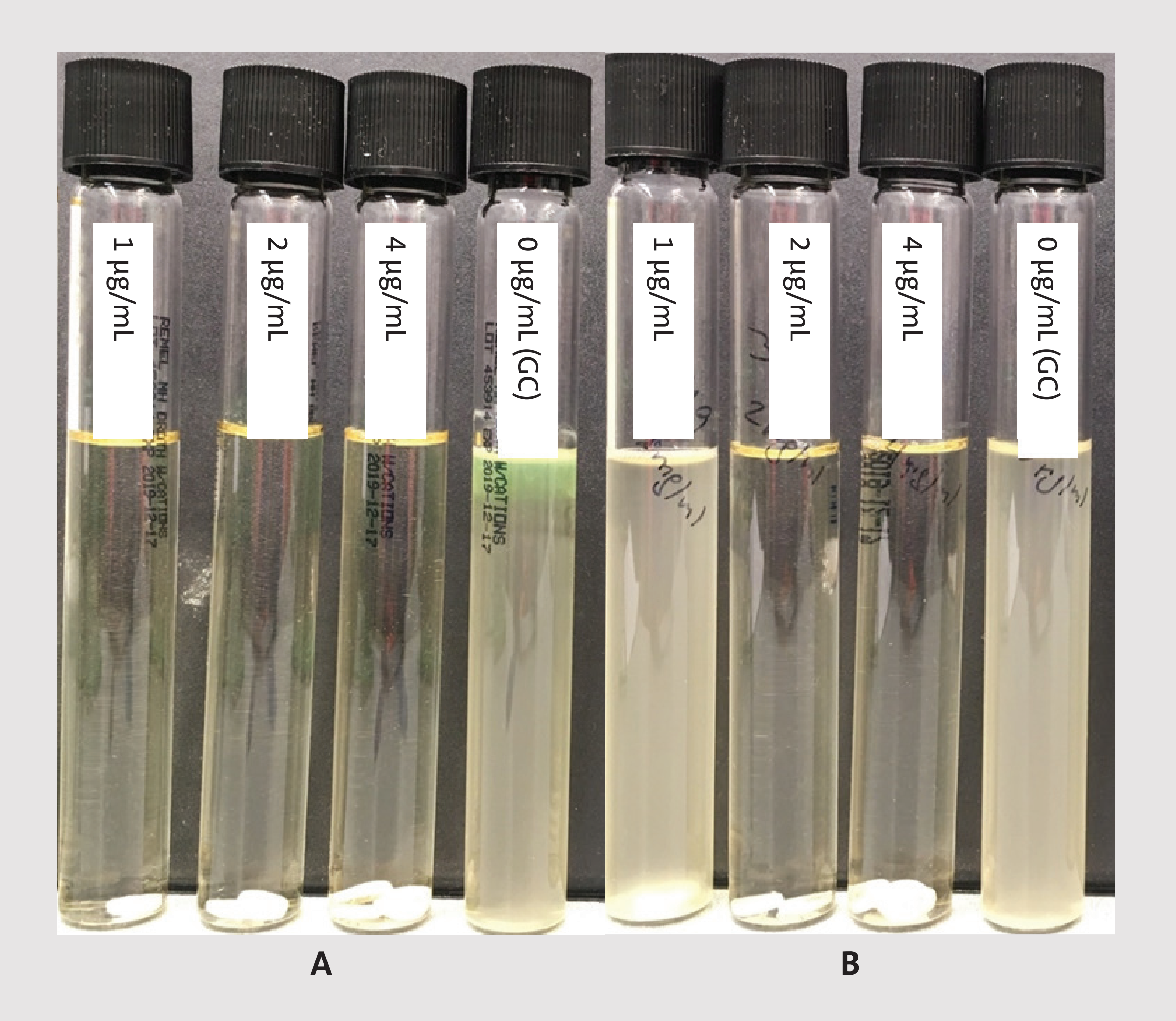
Perform QC of colistin disks and test media daily or **per IQCP** following the routine disk diffusion QC procedure and handle disks as described in CLSI M02.[3]

c. The QC ranges were established with disks (colistin broth disk elution) and media from a limited number of manufacturers and are considered provisional until additional data are evaluated by CLSI and shown to meet CLSI M23[1] guidance.

d. ATCC® is a registered trademark of the American Type Culture Collection. Per ATCC® convention, the trademark symbol is used after "BAA" in each catalog number, in conjunction with the registered ATCC® name.

NOTE: Information in boldface type is new or modified since the previous edition.

Table 3E. (Continued)

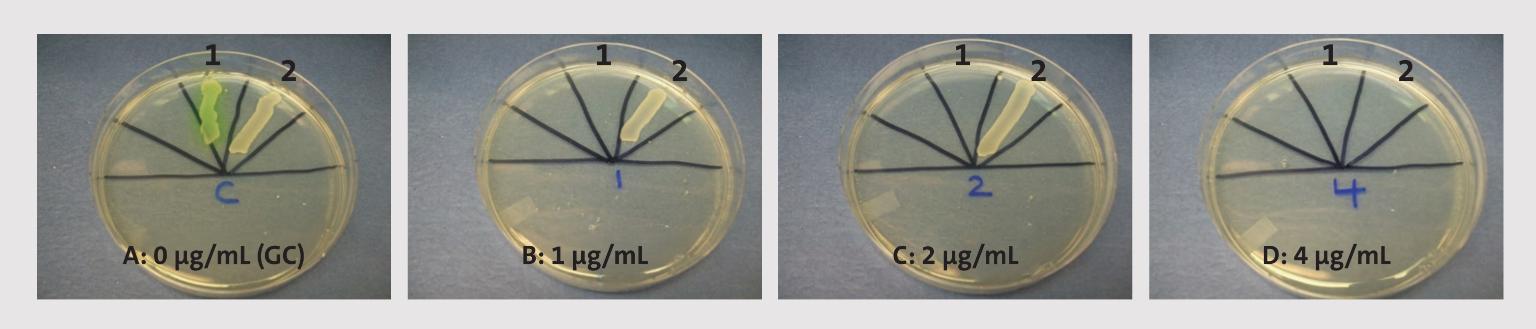


Abbreviations: AR, antimicrobial resistance; ATCC®, American Type Culture Collection; GC, growth control; MIC, minimal inhibitory concentration; QC, quality control.

Figure 1. Colistin Broth Disk Elution.

Results for routine QC strain *P. aeruginosa* ATCC® 27853 with an MIC ≤ 1 µg/mL (A) and supplemental QC strain *E. coli* ATCC® BAA-3170™ (formerly *E. coli* AR Bank #0349 *mcr-1*) with an MIC 2 µg/mL (B).

Table 3E. (Continued)



Abbreviations: AR, antimicrobial resistance; ATCC®, American Type Culture Collection; GC, growth control; MIC, minimal inhibitory concentration; QC, quality control.

Figure 2. Colistin Agar Test.

The plates need to be examined carefully with transmitted light for confluent growth, individual colonies, or light film of growth to determine the MIC. Colistin agar test results for routine QC strain *P. aeruginosa* ATCC® 27853 (position 1) with an MIC ≤ 1 µg/mL and for supplemental QC strain *E. coli* ATCC® BAA-3170™ (formerly *E. coli* AR Bank #0349 *mcr-1*) (position 2) with an MIC 4 µg/mL. The plates shown contain 0 µg/mL (control) (A), 1 µg/mL (B), 2 µg/mL (C), and 4 µg/mL (D) colistin.

References for Table 3E

[1] CLSI. *Development of* In Vitro *Susceptibility Test Methods, Breakpoints, and Quality Control Parameters.* 6th ed. CLSI guideline M23. Clinical and Laboratory Standards Institute; 2023.

[2] CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically.* 12th ed. CLSI standard M07. Clinical and Laboratory Standards Institute; 2024.

[3] CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests.* 14th ed. CLSI standard M02. Clinical and Laboratory Standards Institute; 2024.

Table 3F-1. Test for Performing Disk Diffusion Directly From Positive Blood Culture Broth

Test	Direct Disk Diffusion
Test method	Disk diffusion using positive blood culture broth
Organism group	Enterobacterales, *Pseudomonas aeruginosa*, and *Acinetobacter* spp.
Medium	MHA
Antimicrobial concentration	Standard disk contents for the antimicrobial agents are detailed in Table 3F-2 (Enterobacterales), Table 3F-3 (*P. aeruginosa*), and Table 3F-4 (*Acinetobacter* spp.).
Inoculum	Positive blood culture broth with gram-negative bacilli, used within 8 h of flagging positive by the blood culture system
Test procedure	1. Invert blood culture bottle 5–10 times to thoroughly mix. 2. Sterilize the top of the bottle with an alcohol wipe (allow to dry) and insert 20-gauge venting needle into the blood culture bottle. 3. Dispense 4 drops of blood culture broth onto an MHA plate. As a purity check, use an inoculated blood agar plate streaked for isolation. 4. Spread blood culture broth across the entire surface of the MHA plate using a sterile cotton swab. 5. Repeat this procedure by streaking twice more, rotating the plate approximately 60 degrees each time to ensure an even distribution of inoculum. 6. Leave the lid ajar for 3–5 minutes (ideally) but no more than 15 minutes. 7. Dispense antimicrobial disks onto the surface of the inoculated MHA plate. 8. Press each disk down to ensure complete contact with the agar surface. 9. Invert the plate and place in the incubator within 15 minutes of disks being applied.
Incubation conditions	35°C ± 2°C; ambient air
Incubation length	8–10 h or 16–18 h (refer to Tables 3F-2, 3F-3, and 3F-4 for antimicrobial agent–specific incubation lengths)
Results	1. Examine the blood agar purity plate to ensure pure growth. 2. Examine the test plate to ensure confluent lawn of growth appropriate to read disk zone tests per CLSI M02.[1] 3. Measure the zone diameters according to routine disk diffusion recommendations in CLSI M02.[1] 4. Interpret results using the zone diameter breakpoints in Tables 3F-2, 3F-3, and 3F-4 if the gram-negative bacillus tested is confirmed to be an Enterobacterales, *P. aeruginosa*, or *Acinetobacter* spp., respectively. If species is identified as another organism, do not interpret or report results. 5. Report only the interpretive category and not the measured zone size.

Table 3F-1. (Continued)

Test	Direct Disk Diffusion
Additional testing and reporting	• If there is an inconsistent growth pattern on the plate (eg, mixed inoculum, nonconfluent growth, growth is too faint to read), do not interpret or report results from the direct disk diffusion test, and perform standard susceptibility testing from pure colony growth. • Antimicrobial agents to which the organism is intrinsically resistant (see Appendix B) should be reported as resistant, regardless of measured zone size. • If two zones of growth inhibition are observed, measure the inner zone diameter. In case of colonies present within zones, or presence of both inner and outer zones, check the purity plate and, if pure, record the inner zone diameter.
QC recommendations – routine	• Perform QC according to the standard disk diffusion QC procedures per CLSI M02[1] (eg, daily or **per IQCP**). • See Tables 4A-1 and 4A-2 for acceptable QC ranges.
Supplemental early reading – optional	**• Ranges have been established for early reading (8–10 h) of select QC strain/antimicrobial agent combinations as shown below. This testing is performed using a 0.5 McFarland standardized inoculum (standard disk diffusion QC procedures per CLSI M02[1]).** **• Early reading of QC strains can be used to train staff or assess competency but is not necessary for routine QC.**

Antimicrobial Agent	Disk Content	Optional Early Read (8–10 h) Ranges, mm		
		Escherichia coli ATCC®[a] 25922	*P. aeruginosa* ATCC® 27853	*E. coli* ATCC® 35218
Ampicillin	**10 µg**	**15–22**	**–**	**–**
Ampicillin-sulbactam	**10/10 µg**	**–**	**–**	**13–19**
Ciprofloxacin	**5 µg**	**29–38**	**–**	**–**
Ertapenem	**10 µg**	**–**	**13–21**	**–**
Tobramycin	**10 µg**	**18–26**	**–**	**–**
Trimethoprim-sulfamethoxazole	**1.25/23.75 µg**	**23–29**	**–**	**–**

Table 3F-1. (Continued)

Breakpoint Additions Since 2021	Antimicrobial Agent	Date of Addition (M100 Edition)	8–10 h	16–18 h
	Enterobacterales			
	Ampicillin	March 2021 (M100-Ed31)		X
		March 2023 (M100-Ed33)	X	
	Aztreonam	March 2021 (M100-Ed31)		X
		February 2022 (M100-Ed32)	X	
	Cefepime	**January 2025 (M100-Ed35)**	**X**	**X**
	Ceftazidime	March 2021 (M100-Ed31)		X
		February 2022 (M100-Ed32)	X	
	Ceftriaxone	March 2021 (M100-Ed31)		X
		February 2022 (M100-Ed32)	X	
	Ciprofloxacin	March 2023 (M100-Ed33)	X	X
	Meropenem	March 2023 (M100-Ed33)	X	X
	Tobramycin	March 2021 (M100-Ed31)		X
		February 2022 (M100-Ed32)	X	
	Trimethoprim-sulfamethoxazole	March 2021 (M100-Ed31)		X
	Pseudomonas aeruginosa			
	Cefepime	February 2024 (M100-Ed34)		X
	Ceftazidime	February 2022 (M100-Ed32)		X
		January 2025 (M100-Ed35)	**X**	
	Ciprofloxacin	February 2022 (M100-Ed32)	X	X
	Meropenem	February 2022 (M100-Ed32)		X
		March 2023 (M100-Ed33)	X	
	Tobramycin	February 2022 (M100-Ed32)	X	X
	***Acinetobacter* spp.**			
	Ampicillin-sulbactam	February 2024 (M100-Ed34)		X
		January 2025 (M100-Ed35)	**X**	
	Cefepime	February 2024 (M100-Ed34)	X	X
	Ceftazidime	February 2024 (M100-Ed34)		X
		January 2025 (M100-Ed35)	**X**	

Table 3F-1. (Continued)

Breakpoint Additions Since 2021 (Continued)	Antimicrobial Agent	Date of Addition (M100 Edition)	8–10 h	16–18 h
	***Acinetobacter* spp. (Continued)**			
	Ceftriaxone	February 2024 (M100-Ed34)	X	X
	Ciprofloxacin	February 2024 (M100-Ed34)	X	X
	Meropenem	February 2024 (M100-Ed34)	X	X
	Piperacillin-tazobactam	**January 2025 (M100-Ed35)**	**X**	**X**
	Tobramycin	February 2024 (M100-Ed34)	X	X
	Trimethoprim-sulfamethoxazole	February 2024 (M100-Ed34)	X	X
Breakpoint Revisions Since 2021	**Enterobacterales**			
	Tobramycin	February 2024 (M100-Ed34)	X	X
	Pseudomonas aeruginosa			
	Tobramycin	February 2024 (M100-Ed34)	X	X
	***Acinetobacter* spp.**			
	Ampicillin-sulbactam	**January 2025 (M100-Ed35)**		**X**

Abbreviations: ATCC®, American Type Culture Collection; h, hour(s); **IQCP, individualized quality control plan;** MHA, Mueller-Hinton agar; min, minute(s); QC, quality control.

NOTE: Information in boldface type is new or modified since the previous edition.

Reference for Table 3F-1

[1] CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests*. 14th ed. CLSI standard M02. Clinical and Laboratory Standards Institute; 2024.

Table 3F-2. Zone Diameter Disk Diffusion Breakpoints for Enterobacterales Direct From Blood Culture

General Comments

(1) Organism identification must be known before interpreting and reporting results. Fluoroquinolone breakpoints do not apply to *Salmonella* spp. Aztreonam, ceftazidime, and tobramycin breakpoints do not apply to *Salmonella* or *Shigella* spp.

(2) For additional testing and reporting recommendations, refer to Tables 2A-1 and 2A-2.

NOTE: Information in boldface type is new or modified since the previous edition.

Antimicrobial Agent	Disk Content	Read Times, h	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Comments
			S	I	R	
PENICILLINS						
Ampicillin	10 µg	8–10 16–18	≥ 16 ≥ 17	12–15 14–16	≤ 11 ≤ 13	**(3)** Results of ampicillin testing can be used to predict results for amoxicillin.
CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.)						
Cefepime	**30 µg**	**8–10** **16–18**	**≥ 23** **≥ 23**	**19–22** **19–22**	**≤ 18** **≤ 18**	
Ceftriaxone	30 µg	8–10 16–18	≥ 23 ≥ 23	20–22 20–22	≤ 19 ≤ 19	
Ceftazidime	30 µg	8–10 16–18	≥ 21 ≥ 21	18–20 18–20	≤ 17 ≤ 17	
MONOBACTAMS						
Aztreonam	30 µg	8–10 16–18	≥ 21 ≥ 21	18–20 18–20	≤ 17 ≤ 17	
CARBAPENEMS						
Meropenem	10 µg	8–10 16–18	≥ 22 ≥ 22	20–21 19–21	≤ 19 ≤ 18	
AMINOGLYCOSIDES						
Tobramycin	10 µg	8–10 16–18	≥ 17 ≥ 17	13–16 13–16	≤ 12 ≤ 12	

Table 3F-2. (Continued)

Antimicrobial Agent	Disk Content	Read Times, h	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Comments
			S	I	R	
FLUOROQUINOLONES for Enterobacterales except *Salmonella* spp.						
Ciprofloxacin	5 µg	8–10 16–18	≥ 21 ≥ 21	18–20 18–20	≤ 17 ≤ 17	
FOLATE PATHWAY ANTAGONISTS						
Trimethoprim-sulfamethoxazole	1.25/ 23.75 µg	8–10 16–18	– ≥ 16	– 11–15	– ≤ 10	

Abbreviations: h, hour(s); I, intermediate; R, resistant; S, susceptible.

Table 3F-3. Zone Diameter Disk Diffusion Breakpoints for *Pseudomonas aeruginosa* Direct From Blood Culture

General Comments

(1) Organism identification must be known before interpreting and reporting results.

(2) For additional testing and reporting recommendations, refer to Table 2B-1.

NOTE: Information in boldface type is new or modified since the previous edition.

Antimicrobial Agent	Disk Content	Read Times, h	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Comments
			S	I	R	
CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.)						
Cefepime	30 µg	8–10	–	–	–	**(3)** Confirmatory MIC testing is indicated for isolates with zones of 15–17 mm to avoid reporting false-susceptible or false-resistant results.
		16–18	≥ 18	15–17	≤ 14	
Ceftazidime	30 µg	8–10	**≥ 18**	–	**≤ 14**	**(4) If zone is 15–17 mm at 8–10 h, reincubate and read at 16–18 h.**
		16–18	≥ 18	15–17	≤ 14	
CARBAPENEMS						
Meropenem	10 µg	8–10	≥ 19	16–18	≤ 15	
		16–18	≥ 19	16–18	≤ 15	
AMINOGLYCOSIDES						
Tobramycin	10 µg	8–10	≥ 19	13–18	≤ 12	
		16–18	≥ 19	13–18	≤ 12	
FLUOROQUINOLONES						
Ciprofloxacin	5 µg	8–10	≥ 23	18–22	≤ 17	
		16–18	≥ 25	19–24	≤ 18	

Abbreviations: h, hour(s); I, intermediate; MIC, minimal inhibitory concentration; R, resistant; S, susceptible.

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Table 3F-4. Zone Diameter Disk Diffusion Breakpoints for *Acinetobacter* spp. Direct From Blood Culture

General Comments

(1) Organism identification must be known before interpreting and reporting results.

(2) For additional testing and reporting recommendations, refer to Table 2B-2.

NOTE: Information in boldface type is new or modified since the previous edition.

Antimicrobial Agent	Disk Content	Read Times, h	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Comments
			S	I	R	
β-LACTAM COMBINATION AGENTS						
Ampicillin-sulbactam	10/10 µg	8–10	**≥ 22**	**17–21**	**≤ 16**	
		16–18	**≥ 22**	**17–21**	**≤ 16**	
Piperacillin-tazobactam	**100/10 µg**	**8–10**	**≥ 19**	**17–18**	**≤ 16**	
		16–18	**≥ 19**	**17–18**	**≤ 16**	
CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.)						
Ceftazidime	30 µg	8–10	**≥ 17**	**15–16**	**≤ 14**	
		16–18	≥ 17	15–16	≤ 14	
Cefepime	30 µg	8–10	≥ 18	15–17	≤ 14	
		16–18	≥ 18	15–17	≤ 14	
Ceftriaxone	30 µg	8–10	≥ 21	14–20	≤ 13	
		16–18	≥ 20	13–19	≤ 12	
CARBAPENEMS						
Meropenem	10 µg	8–10	≥ 18	15–17	≤ 14	
		16–18	≥ 18	15–17	≤ 14	
AMINOGLYCOSIDES						
Tobramycin	10 µg	8–10	≥ 15	13–14	≤ 12	
		16–18	≥ 15	13–14	≤ 12	
FLUOROQUINOLONES						
Ciprofloxacin	5 µg	8–10	≥ 21	16–20	≤ 15	
		16–18	≥ 21	16–20	≤ 15	

Table 3F-4. (Continued)

Antimicrobial Agent	Disk Content	Read Times, h	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Comments
			S	I	R	
FOLATE PATHWAY ANTAGONISTS						
Trimethoprim-sulfamethoxazole	1.25/23.75 µg	8–10	≥ 16	11–15	≤ 10	
		16–18	≥ 16	11–15	≤ 10	

Abbreviations: h, hour(s); I, intermediate; R, resistant; S, susceptible.

Table 3G. Tests for Detecting β-Lactamase Production in *Staphylococcus* spp.

Test	**β-Lactamase Production**	
Test method	Disk diffusion (penicillin zone-edge test)	Nitrocefin-based test
Organism group	*S. aureus* with penicillin MICs ≤ 0.12 µg/mL or zones ≥ 29 mm[a]	*Staphylococcus* spp.[a,b] with penicillin MICs ≤ 0.12 µg/mL or zones ≥ 29 mm
Medium	MHA	N/A
Antimicrobial concentration	10 units penicillin disk	N/A
Inoculum	Standard disk diffusion procedure	Induced growth (ie, growth taken from the zone margin surrounding a penicillin or cefoxitin disk test on either MHA or a blood agar plate after 16–18 h of incubation)
Incubation conditions	35°C ± 2°C; ambient air	Room temperature
Incubation length	16–18 h	Up to 1 h for nitrocefin-based test or follow manufacturer's directions
Results	Sharp zone edge ("cliff") = β-lactamase positive (see Figure 1 below this table) Fuzzy zone edge ("beach") = β-lactamase negative (see Figure 2 below this table)	Nitrocefin-based test: conversion from yellow to red/pink = β-lactamase positive
Additional testing and reporting	β-Lactamase–positive staphylococci are resistant to penicillin, amino-, carboxy-, and ureidopenicillins.	Nitrocefin-based tests can be used for *S. aureus*, but negative results should be confirmed with the penicillin zone-edge test before penicillin is reported as susceptible. β-Lactamase–positive staphylococci are resistant to penicillin, amino-, carboxy-, and ureidopenicillins.
QC recommendations – routine[c]	*S. aureus* ATCC®[d] 25923 for routine QC of penicillin disk to include examination of zone-edge test (fuzzy edge = "beach")	
QC recommendations – lot/shipment[e]		*S. aureus* ATCC® 29213 – positive *S. aureus* ATCC® 25923 – negative (or see local regulations and manufacturers' recommendations)
QC recommendations – supplemental[f]	*S. aureus* ATCC® 29213 – positive penicillin zone-edge test (sharp edge = "cliff")	

Abbreviations: AST, antimicrobial susceptibility testing; ATCC®, American Type Culture Collection; h, hour(s); **IQCP, individualized quality control plan;** MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; N/A, not applicable; QC, quality control.

Table 3G. (Continued)

Footnotes

a. The penicillin disk diffusion zone-edge test was shown to be more sensitive than nitrocefin-based tests for detection of β-lactamase production in *S. aureus*. The penicillin zone-edge test is recommended if only one test is used for β-lactamase detection. However, some laboratories may choose to perform a nitrocefin-based test first and, if this test is positive, report the results as positive for β-lactamase (or penicillin resistant). If the nitrocefin test is negative, the penicillin zone-edge test should be performed before reporting the isolate as penicillin susceptible in cases in which penicillin may be used for therapy (eg, endocarditis).[1,2]

b. For *S. lugdunensis*, tests for β-lactamase detection are not necessary because isolates producing a β-lactamase will test penicillin resistant (MIC > 0.12 µg/mL and zone diameters < 29 mm). If a laboratory is using a method other than the CLSI disk diffusion or MIC reference methods and is unsure if the method can reliably detect penicillin resistance with contemporary isolates of *S. lugdunensis,* the laboratory should perform an induced nitrocefin assay or other CLSI reference method on isolates that test penicillin susceptible before reporting the isolate as penicillin susceptible.

c. QC recommendations – routine

Test negative (susceptible) QC strain:

- With each new lot/shipment of testing materials
- Daily if the test is performed less than once per week and/or **an IQCP justifying less frequent QC has not been developed**
- **Less frequent than daily if the test is performed at least once per week and an IQCP has been developed**

d. ATCC® is a registered trademark of the American Type Culture Collection.

e. QC recommendations – lot/shipment

Test positive (resistant) QC strain at minimum with each new lot/shipment of testing materials.

f. QC recommendations – supplemental

- Supplemental QC strains can be used to assess a new test, for training personnel, and for competence assessment. It is not necessary to include supplemental QC strains in routine AST QC programs. See Appendix C, which describes use of QC strains.

NOTE: Information in boldface type is new or modified since the previous edition.

Table 3G. (Continued)

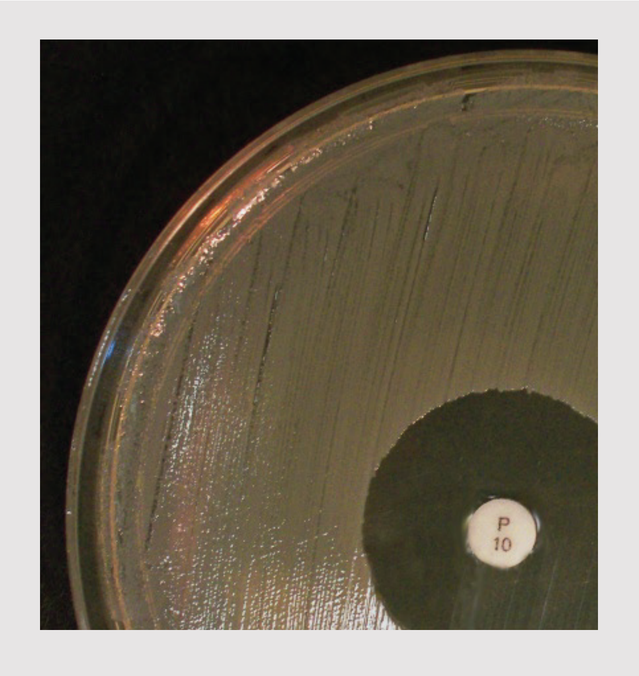

Figure 1. Positive Penicillin Disk Zone-Edge Test for β-Lactamase Detection.

The zone edge is sharp or like a “cliff” indicating β-lactamase production.

Table 3G. (Continued)

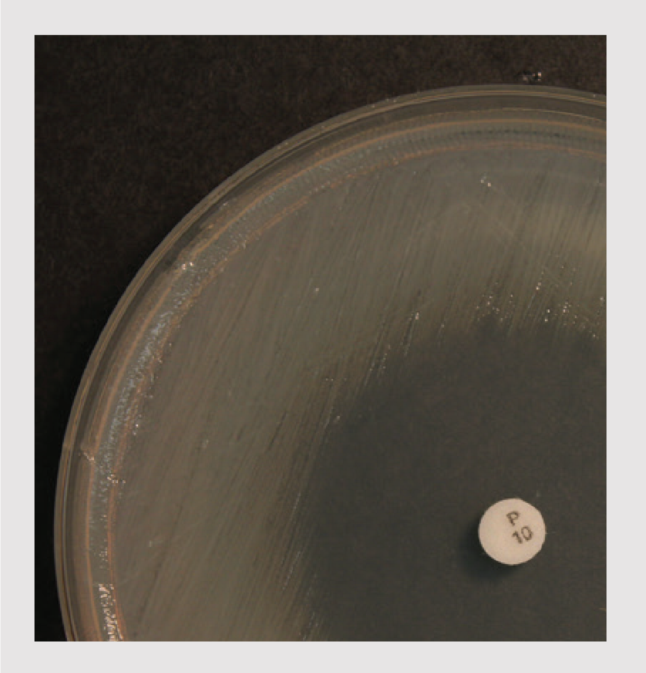

Figure 2. Negative Penicillin Disk Zone-Edge Test for β-Lactamase Detection.

The zone edge is fuzzy or like a "beach," indicating no β-lactamase production.

References for Table 3G

[1] Kaase M, Lenga S, Friedrich S, et al. Comparison of phenotypic methods for penicillinase detection in *Staphylococcus aureus*. *Clin Microbiol Infect*. 2008;14(6):614-616.

[2] Gill VJ, Manning CB, Ingalls CM. Correlation of penicillin minimum inhibitory concentrations and penicillin zone edge appearance with staphylococcal beta-lactamase production. *J Clin Microbiol*. 1981;14(4):437-440.

Table 3H. Oxacillin Salt Agar Test for Detecting Methicillin (Oxacillin) Resistance in *Staphylococcus aureus*[a]

Test	Oxacillin Salt Agar
Test method	Agar dilution
Medium	MHA with 4% NaCl
Antimicrobial concentration	6 µg/mL oxacillin
Inoculum	Colony suspension to obtain 0.5 McFarland turbidity Using a 1-µL loop that was dipped in the suspension, spot an area 10–15 mm in diameter. Alternatively, using a swab dipped in the suspension and the excess liquid expressed, spot a similar area or streak an entire quadrant.
Incubation conditions	33 to 35°C; ambient air[b]
Incubation length	24 h; read with transmitted light
Results	Examine carefully with transmitted light. > 1 colony or light film of growth = methicillin (oxacillin) resistant
Additional testing and reporting	MRS are resistant to currently available β-lactam agents, except ceftaroline, and these agents should be reported as resistant or should not be reported.[c]
QC recommendations – routine[d]	*S. aureus* ATCC®[e] 29213 – methicillin (oxacillin) susceptible (≤ 1 colony)
QC recommendations – lot/shipment[f]	*S. aureus* ATCC® 43300 – methicillin (oxacillin) resistant (> 1 colony)

Abbreviations: ATCC®, American Type Culture Collection; h, hour(s); **IQCP, individualized quality control plan**; MHA, Mueller-Hinton agar; MRS, methicillin (oxacillin)-resistant *Staphylococcus* spp.; NaCl, sodium chloride; QC, quality control.

Table 3H. (Continued)

Footnotes

a. Including members of the *S. aureus* complex (see Table 2C, general comment [3]).

b. Testing at temperatures above 35°C may not detect MRS.

c. Testing of other β-lactam agents, except ceftaroline, is not advised.

d. QC recommendations – routine

Test negative (susceptible) QC strain:

- With each new lot/shipment of testing materials
- Daily if the test is performed less than once per week and/or a**n IQCP justifying less frequent QC has not been developed**
- **Less frequent than daily if the test is performed at least once per week and an IQCP has been developed**

e. ATCC® is a registered trademark of the American Type Culture Collection.

f. QC Recommendations – lot/shipment

Test positive (resistant) QC strain at minimum with each new lot/shipment of testing materials.

NOTE: Information in boldface type is new or modified since the previous edition.

Table 3I. Vancomycin Agar Screen for *Staphylococcus aureus* and *Enterococcus* spp.

Screen Test	**Vancomycin MIC ≥ 8 µg/mL**	
Test method	Agar dilution	Agar dilution
Organism group	*S. aureus*	*Enterococcus* spp.
Medium	BHI agar	BHI[a] agar
Antimicrobial concentration	6 µg/mL vancomycin	6 µg/mL vancomycin
Inoculum	Colony suspension to obtain 0.5 McFarland turbidity Preferably, using a micropipette, spot a 10-µL drop onto agar surface. Alternatively, using a swab dipped in the suspension and the excess liquid expressed, spot an area 10–15 mm in diameter or streak a portion of the plate.	1–10 µL of a 0.5 McFarland suspension spotted onto agar surface. Alternatively, using a swab dipped in the suspension and the excess liquid expressed, spot an area 10–15 mm in diameter or streak a portion of the plate.
Incubation conditions	35°C ± 2°C; ambient air	35°C ± 2°C; ambient air
Incubation length	24 h	24 h
Results	Examine carefully with transmitted light for > 1 colony or light film of growth. > 1 colony = presumptive reduced susceptibility to vancomycin	> 1 colony = presumptive vancomycin resistance
Additional testing and reporting	Perform a vancomycin MIC using a validated MIC method to determine vancomycin MICs on *S. aureus* that grow on BHI–vancomycin screening agar. Testing on BHI–vancomycin screening agar does not reliably detect all vancomycin-intermediate *S. aureus* strains. Some strains for which the vancomycin MICs are 4 µg/mL will fail to grow.	Perform vancomycin MIC on *Enterococcus* spp. that grow on BHI–vancomycin screening agar and test for motility and pigment production to distinguish species with acquired resistance (eg, *vanA* and *vanB*) from those with intrinsic, intermediate-level resistance to vancomycin (eg, *vanC*), such as *E. gallinarum* and *E. casseliflavus,* which often grow on the vancomycin screen plate. In contrast to other enterococci, *E. casseliflavus* and *E. gallinarum* with vancomycin MICs of 8–16 µg/mL (intermediate) differ from VRE for infection prevention purposes.
QC recommendations – routine[b]	*E. faecalis* ATCC®[c] 29212 – susceptible	*E. faecalis* ATCC® 29212 – susceptible
QC recommendations – lot/shipment[d]	*E. faecalis* ATCC® 51299 – resistant	*E. faecalis* ATCC® 51299 – resistant

Abbreviations: ATCC®, American Type Culture Collection; BHI, brain heart infusion; h, hour(s); **IQCP, individualized quality control plan;** MIC, minimal inhibitory concentration; QC, quality control; VRE, vancomycin-resistant enterococci.

Table 3I. (Continued)

Footnotes

a. Even though not as widely available, dextrose phosphate agar and broth have been shown in limited testing to perform comparably with BHI media.

b. QC recommendations – routine

Test negative (susceptible) QC strain:

- With each new lot/shipment of testing materials
- Daily if the test is performed less than once per week and/or **an IQCP justifying less frequent QC has not been developed**
- **Less frequent than daily if the test is performed at least once per week and an IQCP has been developed**

c. ATCC® is a registered trademark of the American Type Culture Collection.

d. QC recommendations – lot/shipment

Test positive (resistant) QC strain at minimum with each new lot/shipment of testing materials.

NOTE: Information in boldface type is new or modified since the previous edition.

Table 3J. Tests for Detecting Inducible Clindamycin Resistance in *Staphylococcus* spp., *Streptococcus pneumoniae*, and *Streptococcus* spp. β-Hemolytic Group[a,b]

Test	ICR			
Test method	**Disk Diffusion (D-zone test)**		**Broth Microdilution**	
Organism group (applies only to organisms resistant to erythromycin and susceptible or intermediate to clindamycin)	All *Staphylococcus* spp.	*S. pneumoniae* and β-hemolytic *Streptococcus* spp.	All *Staphylococcus* spp.[c]	*S. pneumoniae* and β-hemolytic *Streptococcus* spp.
Medium	MHA or blood agar purity plate used with MIC tests	MHA supplemented with sheep blood (5% v/v) or TSA supplemented with sheep blood (5% v/v)	CAMHB	CAMHB with LHB (2.5% to 5% v/v)
Antimicrobial concentration	15-µg erythromycin and 2-µg clindamycin disks spaced 15–26 mm apart (edge-to-edge)	15-µg erythromycin and 2-µg clindamycin disks spaced 12 mm apart (edge-to-edge)	4 µg/mL erythromycin and 0.5 µg/mL clindamycin in same well	1 µg/mL erythromycin and 0.5 µg/mL clindamycin in same well
Inoculum	Standard disk diffusion procedure or heavily inoculated area of purity plate	Standard disk diffusion procedure	Standard broth microdilution procedure	
Incubation conditions	35°C ± 2°C; ambient air	35°C ± 2°C; 5% CO_2	35°C ± 2°C; ambient air	
Incubation length	16–18 h	20–24 h	18–24 h	20–24 h
Results	Flattening of the zone of inhibition adjacent to the erythromycin disk (referred to as a D-zone) = ICR. Hazy growth within the zone of inhibition around clindamycin = clindamycin resistance, even if no D-zone is apparent.		Any growth = ICR. No growth = no ICR.	
Additional testing and reporting	Report isolates with ICR as “clindamycin resistant.” The following comment may be included with the report: “This isolate is presumed to be resistant based on detection of ICR, as determined by testing clindamycin in combination with erythromycin.”			
QC recommendations – routine[c]	*Staphylococcus aureus* ATCC®[d] 25923 for routine QC of erythromycin and clindamycin disks	*S. pneumoniae* ATCC® 49619 for routine QC of erythromycin and clindamycin disks	*S. aureus* ATCC® BAA-976™ or *S. aureus* ATCC® 29213 – no growth	*S. pneumoniae* ATCC® 49619 or *S. aureus* ATCC® BAA-976™ – no growth

Table 3J. (Continued)

Test	ICR	
Test method	**Disk Diffusion (D-zone test)**	**Broth Microdilution**
QC recommendations – lot/ shipment[e]	Perform QC according to standard disk diffusion QC procedures per CLSI M02[1] (eg, daily or **per IQCP**).	*S. aureus* ATCC® BAA-977™ – growth
QC recommendations – supplemental[f]	*S. aureus* ATCC® BAA-976™ (D-zone test negative) *S. aureus* ATCC® BAA-977™ (D-zone test positive) Use of unsupplemented MHA is acceptable for these strains.	*S. aureus* ATCC® BAA-976™ (no growth) *S. aureus* ATCC® BAA-977™ (growth)

Abbreviations: AST, antimicrobial susceptibility testing; ATCC®, American Type Culture Collection; CAMHB, cation-adjusted Mueller-Hinton broth; CO_2, carbon dioxide; h, hour(s); ICR, inducible clindamycin resistance; **IQCP, individualized quality control plan;** LHB, lysed horse blood; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; QC, quality control; TSA, tryptic soy agar.

Footnotes

a. AST of β-hemolytic streptococci does not need to be performed routinely (see general comment [5] in Table 2H-1). When susceptibility testing is clinically indicated, test for ICR in strains that are erythromycin resistant and clindamycin susceptible or intermediate.

b. In accordance with current guidance from the American College of Obstetricians and Gynecologists,[2] colonizing isolates of group B streptococci from severe penicillin-allergic pregnant women should be tested for clindamycin (including ICR) (see comment [12] in Table 2H-1).[2] For isolates that test susceptible to clindamycin (with erythromycin induction), consider adding the following comment to the patient's report: "For intrapartum prophylaxis, this group B *Streptococcus* does not demonstrate ICR as determined by testing clindamycin in combination with erythromycin."

c. QC recommendations – routine

Test negative (susceptible) QC strain:

- With each new lot/shipment of testing materials
- Daily if the test is performed less than once per week and/or **an IQCP justifying less frequent QC has not been developed**
- **Less frequent than daily if the test is performed at least once per week and an IQCP has been developed**

d. ATCC® is a registered trademark of the American Type Culture Collection. Per ATCC® convention, the trademark symbol is used after "BAA" in each catalog number, in conjunction with the registered ATCC® name.

e. QC recommendations – lot/shipment

Test positive (resistant) QC strain at minimum with each new lot/shipment of testing materials.

Table 3J. (Continued)

f. QC recommendations – supplemental

- Supplemental QC strains can be used to assess a new test, for training personnel, and for competence assessment. It is not necessary to include supplemental QC strains in routine AST QC programs. See Appendix C, which describes use of QC strains.

NOTE: Information in boldface type is new or modified since the previous edition.

References for Table 3J

[1] CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests*. 14th ed. CLSI standard M02. Clinical and Laboratory Standards Institute; 2024.

[2] American College of Obstetricians and Gynecologists. Prevention of group B streptococcal early-onset disease in newborns: ACOG Committee Opinion, Number 797. *Obstet Gynecol*. 2020;135(2):e51-e72. doi:10.1097/AOG.0000000000003668

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Table 3K. Test for Detecting High-Level Mupirocin Resistance in *Staphylococcus aureus*

Test	High-Level Mupirocin Resistance[a,1-3]	
Test method	Disk diffusion	Broth microdilution
Organism group	*S. aureus*	
Medium	MHA	CAMHB
Antimicrobial concentration	200-µg mupirocin disk	Single mupirocin 256-µg/mL well
Inoculum	Standard disk diffusion procedure	Standard broth microdilution procedure
Incubation conditions	35°C ± 2°C; ambient air	35°C ± 2°C; ambient air
Incubation length	24 h; read with transmitted light	24 h
Results	Examine carefully with transmitted light for light growth within the zone of inhibition. No zone = high-level mupirocin resistance. Any zone = the absence of high-level mupirocin resistance.	For single 256-µg/mL well: Growth = high-level mupirocin resistance. No growth = the absence of high-level mupirocin resistance.
Additional testing and reporting	Report isolates with no zone as high-level mupirocin resistant. Report any zone of inhibition as the absence of high-level resistance.	Report growth in the 256-µg/mL well as high-level mupirocin resistant. Report no growth in the 256-µg/mL well as the absence of high-level resistance.
QC recommendations – routine[b]	*S. aureus* ATCC®[c] 25923 (200-µg disk) – *mupA* negative (zone 29–38 mm)	*S. aureus* ATCC® 29213 – *mupA* negative (MIC 0.06–0.5 µg/mL) or *Enterococcus faecalis* ATCC® 29212 – *mupA* negative (MIC 16–128 µg/mL)
QC recommendations – lot/ shipment[d]	*S. aureus* ATCC® BAA-1708™ – *mupA* positive (no zone)	*S. aureus* ATCC® BAA-1708™ – *mupA* positive (growth in 256-µg/mL well)

Abbreviations: ATCC®, American Type Culture Collection; CAMHB, cation-adjusted Mueller-Hinton broth; h, hour(s); **IQCP, individualized quality control plan;** MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; QC, quality control.

Footnotes

a. Although not formally validated by CLSI M23[1]–based analyses, some studies have linked a lack of response to mupirocin-based decolonization regimens with isolates for which the mupirocin MICs are ≥ 512 µg/mL.[2-4] Although CLSI M100 does not provide guidance on breakpoints for mupirocin, disk-based testing and the MIC test described here identify isolates for which the mupirocin MICs are ≥ 512 µg/mL.

Table 3K. (Continued)

b. QC recommendations – routine

Test negative (susceptible) QC strain:

- With each new lot/shipment of testing materials
- Daily if the test is performed less than once per week and/or if **an IQCP justifying less frequent QC has not been developed**
- **Less frequent than daily if the test is performed at least once per week and an IQCP has been developed**

c. ATCC® is a registered trademark of the American Type Culture Collection. Per ATCC® convention, the trademark symbol is used after "BAA" in each catalog number, in conjunction with the registered ATCC® name.

d. QC recommendations – lot/shipment

Test positive (resistant) QC strain at minimum with each new lot/shipment of testing materials.

NOTE: Information in boldface type is new or modified since the previous edition.

References for Table 3K

[1] CLSI. *Development of* In Vitro *Susceptibility Test Methods, Breakpoints, and Quality Control Parameters.* 6th ed. CLSI guideline M23. Clinical and Laboratory Standards Institute; 2023.

[2] Simor AE, Phillips E, McGeer A, et al. Randomized controlled trial of chlorhexidine gluconate for washing, intranasal mupirocin, and rifampin and doxycycline versus no treatment for the eradication of methicillin-resistant *Staphylococcus aureus* colonization. *Clin Infect Dis.* 2007;44(2):178-185. doi:10.1086/510392

[3] Harbarth S, Dharan S, Liassine N, Herrault P, Auckenthaler R, Pittet D. Randomized, placebo-controlled, double-blind trial to evaluate the efficacy of mupirocin for eradicating carriage of methicillin-resistant *Staphylococcus aureus. Antimicrob Agents Chemother.* 1999;43(6):1412-1416. doi:10.1128/AAC.43.6.1412

[4] Walker ES, Vasquez JE, Dula R, Bullock H, Sarubbi FA. Mupirocin-resistant, methicillin-resistant *Staphylococcus aureus:* does mupirocin remain effective? *Infect Control Hosp Epidemiol.* 2003;24(5):342-346. doi:10.1086/502218

Table 3L. Test for Detecting High-Level Aminoglycoside Resistance in *Enterococcus* spp.[a] (including disk diffusion)

Test	**Gentamicin HLAR**			**Streptomycin HLAR**		
Test method	Disk diffusion	Broth microdilution	Agar dilution	Disk diffusion	Broth microdilution	Agar dilution
Medium	MHA	BHI[b] broth	BHI[b] agar	MHA	BHI[b] broth	BHI[b] agar
Antimicrobial concentration	120-µg gentamicin disk	Gentamicin, 500 µg/mL	Gentamicin, 500 µg/mL	300-µg streptomycin disk	Streptomycin, 1000 µg/mL	Streptomycin, 2000 µg/mL
Inoculum	Standard disk diffusion procedure	Standard broth dilution procedure	10 µL of a 0.5 McFarland suspension spotted onto agar surface	Standard disk diffusion procedure	Standard broth dilution procedure	10 µL of a 0.5 McFarland suspension spotted onto agar surface
Incubation conditions	35°C ± 2°C; ambient air	35°C ± 2°C; ambient air	35°C ± 2°C; ambient air	35°C ± 2°C; ambient air	35°C ± 2°C; ambient air	35°C ± 2°C; ambient air
Incubation length	16–18 h	24 h	24 h	16–18 h	24–48 h (if susceptible at 24 h, reincubate)	24–48 h (if susceptible at 24 h, reincubate)
Results	6 mm = resistant 7–9 mm = inconclusive ≥ 10 mm = susceptible MIC correlates: R = > 500 µg/mL S = ≤ 500 µg/mL	Any growth = resistant	> 1 colony = resistant	6 mm = resistant 7–9 mm = inconclusive ≥ 10 mm = susceptible MIC correlates: R = > 1000 µg/mL (broth) and > 2000 µg/mL (agar) S = ≤ 1000 µg/mL (broth) and ≤ 2000 µg/mL (agar)	Any growth = resistant	> 1 colony = resistant

Table 3L. (Continued)

Test	Gentamicin HLAR			Streptomycin HLAR		
Additional testing and reporting	Resistant: is not synergistic with cell wall–active agent (eg, ampicillin, penicillin, and vancomycin). Susceptible: is synergistic with cell wall–active agent (eg, ampicillin, penicillin, and vancomycin) that is also susceptible. If disk diffusion result is inconclusive: perform an agar dilution or broth dilution MIC test to confirm. Strains of enterococci with ampicillin and penicillin MICs ≥ 16 µg/mL are categorized as resistant. However, enterococci with penicillin MICs ≤ 64 µg/mL or ampicillin MICs ≤ 32 µg/mL may be susceptible to synergistic killing by these penicillins in combination with gentamicin or streptomycin (in the absence of high-level resistance to gentamicin or streptomycin, see CLSI M07[1]) if high doses of penicillin or ampicillin are used. Enterococci possessing higher levels of penicillin (MICs ≥ 128 µg/mL) or ampicillin (MICs ≥ 64 µg/mL) resistance may not be susceptible to the synergistic effect.[2,3] Physicians' requests to determine the actual MIC of penicillin or ampicillin for blood and CSF isolates of enterococci should be considered.					
QC recommendations – routine[c]	*E. faecalis* ATCC®[d] 29212: 16–23 mm	*E. faecalis* ATCC® 29212 – susceptible	*E. faecalis* ATCC® 29212 – susceptible	*E. faecalis* ATCC® 29212: 14–20 mm	*E. faecalis* ATCC® 29212 – susceptible	*E. faecalis* ATCC® 29212 – susceptible
QC recommendations – lot/shipment[e]		*E. faecalis* ATCC® 51299 – resistant	*E. faecalis* ATCC® 51299 – resistant		*E. faecalis* ATCC® 51299 – resistant	*E. faecalis* ATCC® 51299 – resistant

Abbreviations: ATCC®, American Type Culture Collection; BHI, brain heart infusion; CSF, cerebrospinal fluid; h, hour(s); HLAR, high-level aminoglycoside resistance; **IQCP, individualized quality control plan;** MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; QC, quality control; R, resistant; S, susceptible.

Footnotes

a. Other aminoglycosides do not need to be tested, because their activities against enterococci are not superior to gentamicin and streptomycin.

b. Even though not as widely available, dextrose phosphate agar and broth have been shown in limited testing to perform comparably with BHI media.

c. QC recommendations – routine

Test negative (susceptible) QC strain:

- With each new lot/shipment of testing materials
- Daily if the test is performed less than once per week and/or if **an IQCP justifying less frequent QC has not been developed**
- **Less frequent than daily if the test is performed at least once per week and an IQCP has been developed**

d. ATCC® is a registered trademark of the American Type Culture Collection.

e. QC recommendations – lot/shipment

Test positive (resistant) QC strain at minimum with each new lot/shipment of testing materials.

NOTE: Information in boldface type is new or modified since the previous edition.

Table 3L. (Continued)

References for Table 3L

[1] CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*. 12th ed. CLSI standard M07. Clinical and Laboratory Standards Institute; 2024.

[2] Torres C, Tenorio C, Lantero M, Gastañares MJ, Baquero F. High-level penicillin resistance and penicillin-gentamicin synergy in *Enterococcus faecium*. *Antimicrob Agents Chemother*. 1993;37(11):2427-2431. doi:10.1128/AAC.37.11.2427

[3] Murray BE. Vancomycin-resistant enterococci. *Am J Med*. 1997;102(3):284-293. doi:10.1016/S0002-9343(99)80270-8

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Table 4A-1. Disk Diffusion QC Ranges for Nonfastidious Organisms and Antimicrobial Agents Excluding β-Lactam Combination Agents[a]

Antimicrobial Agent	Disk Content	Disk Diffusion QC Ranges, mm		
		Escherichia coli ATCC®[b] 25922	*Pseudomonas aeruginosa* ATCC® 27853	*Staphylococcus aureus* ATCC® 25923
Amikacin	30 µg	19–26	20–26	20–26
Ampicillin	10 µg	15–22	–	27–35
Azithromycin	15 µg	–	–	21–26
Azlocillin	75 µg	–	24–30	–
Aztreonam	30 µg	28–36	23–29	–
Carbenicillin	100 µg	23–29	18–24	–
Cefaclor	30 µg	23–27	–	27–31
Cefamandole	30 µg	26–32	–	26–34
Cefazolin	30 µg	21–27	–	29–35
Cefdinir	5 µg	24–28	–	25–32
Cefditoren	5 µg	22–28	–	20–28
Cefepime	30 µg	31–37	25–31	23–29
Cefetamet	10 µg	24–29	–	–
Cefiderocol	30 µg	25–31	22–31	–
Cefixime	5 µg	20–26	–	–
Cefmetazole	30 µg	26–32	–	25–34
Cefonicid	30 µg	25–29	–	22–28
Cefoperazone	75 µg	28–34	23–29	24–33
Cefotaxime	30 µg	29–35	18–22	25–31
Cefotetan	30 µg	28–34	–	17–23
Cefoxitin[c]	30 µg	23–29	–	23–29
Cefpodoxime	10 µg	23–28	–	19–25
Cefprozil	30 µg	21–27	–	27–33
Ceftaroline	30 µg	26–34	–	26–35
Ceftazidime	30 µg	25–32	22–29	16–20

Table 4A-1. (Continued)

Antimicrobial Agent	Disk Content	Disk Diffusion QC Ranges, mm		
		Escherichia coli ATCC®[b] 25922	*Pseudomonas aeruginosa* ATCC® 27853	*Staphylococcus aureus* ATCC® 25923
Ceftibuten	30 µg	27–35	–	–
Ceftizoxime	30 µg	30–36	12–17	27–35
Ceftobiprole	5 µg	25–31	–	20–27
Ceftriaxone	30 µg	29–35	17–23	22–28
Cefuroxime	30 µg	20–26	–	27–35
Cephalothin	30 µg	15–21	–	29–37
Chloramphenicol	30 µg	21–27	–	19–26
Cinoxacin	100 µg	26–32	–	–
Ciprofloxacin	5 µg	29–38	25–33	22–30
Clarithromycin	15 µg	–	–	26–32
Clinafloxacin	5 µg	31–40	27–35	28–37
Clindamycin[d]	2 µg	–	–	24–30
Colistin	10 µg	11–17	11–17	–
Delafloxacin[e]	5 µg	28–35	23–29	32–40
Dirithromycin	15 µg	–	–	18–26
Doripenem	10 µg	27–35	28–35	33–42
Doxycycline	30 µg	18–24	–	23–29
Enoxacin	10 µg	28–36	22–28	22–28
Eravacycline	20 µg	17–24	–	19–26
Ertapenem	10 µg	29–36	13–21	24–31
Erythromycin[d]	15 µg	–	–	22–30
Faropenem	5 µg	20–26	–	27–34
Fleroxacin	5 µg	28–34	12–20	21–27
Fosfomycin[f]	200 µg	22–30	–	25–33
Fusidic acid	10 µg	–	–	24–32
Garenoxacin	5 µg	28–35	19–25	30–36
Gatifloxacin	5 µg	30–37	20–28	27–33

Table 4A-1. (Continued)

Antimicrobial Agent	Disk Content	Disk Diffusion QC Ranges, mm		
		Escherichia coli ATCC®[b] 25922	*Pseudomonas aeruginosa* ATCC® 27853	*Staphylococcus aureus* ATCC® 25923
Gemifloxacin	5 µg	29–36	19–25	27–33
Gentamicin[g]	10 µg	19–26	17–23	19–27
Gepotidacin	10 µg	18–26	–	23–29
Grepafloxacin	5 µg	28–36	20–27	26–31
Iclaprim	5 µg	14–22	–	25–33
Imipenem[h]	10 µg	26–32	20–28	–
Kanamycin	30 µg	17–25	–	19–26
Lefamulin	20 µg	–	–	26–32
Levofloxacin	5 µg	29–37	19–26	25–30
Levonadifloxacin	10 µg	27–33[e]	17–23[e]	32–39[e]
Linezolid	30 µg	–	–	24–30
Lomefloxacin	10 µg	27–33	22–28	23–29
Loracarbef	30 µg	23–29	–	23–31
Mecillinam	10 µg	24–30	–	–
Meropenem	10 µg	28–35	27–33	29–37
Minocycline	30 µg	**20–26**	–	25–30
Moxalactam	30 µg	28–35	17–25	18–24
Moxifloxacin	5 µg	28–35	17–25	28–35
Nafcillin	1 µg	–	–	16–22
Nafithromycin	15 µg	–	–	25–31[e]
Nalidixic acid	30 µg	22–28	–	–
Netilmicin	30 µg	22–30	17–23	22–31
Nitrofurantoin	300 µg	20–25	–	18–22
Norfloxacin	10 µg	28–35	22–29	17–28
Ofloxacin	5 µg	29–33	17–21	24–28
Omadacycline	30 µg	22–28	–	22–30
Oxacillin	1 µg	–	–	18–24

Table 4A-1. (Continued)

Antimicrobial Agent	Disk Content	Disk Diffusion QC Ranges, mm		
		Escherichia coli ATCC®[b] 25922	*Pseudomonas aeruginosa* ATCC® 27853	*Staphylococcus aureus* ATCC® 25923
Pefloxacin	5 µg	25–33	–	–
Penicillin	10 units	–	–	26–37
Piperacillin	100 µg	24–30	25–33	–
Plazomicin	30 µg	21–27	15–21	19–25
Polymyxin B	300 units	13–19	14–18	–
Quinupristin-dalfopristin	15 µg	–	–	21–28
Razupenem	10 µg	21–26	–	–[i]
Rifampin	5 µg	8–10	–	26–34
Solithromycin	15 µg	–	–	22–30
Sparfloxacin	5 µg	30–38	21–29	27–33
Streptomycin[g]	10 µg	12–20	–	14–22
Sulfisoxazole[j,k]	250 µg or 300 µg	15–23	–	24–34
Sulopenem	2 µg	24–30[e]	–	–
Tebipenem[h]	10 µg	30–37	20–26	–
Tedizolid[l]	2 µg	–	–	19–25
Teicoplanin	30 µg	–	–	15–21
Telithromycin	15 µg	–	–	24–30
Tetracycline	30 µg	18–25	–	24–30
Ticarcillin	75 µg	24–30	21–27	–
Tigecycline	15 µg	20–27	9–13	20–25
Tobramycin	10 µg	18–26	20–26	19–29
Trimethoprim[j]	5 µg	21–28	–	19–26
Trimethoprim-sulfamethoxazole[j]	1.25/23.75 µg	23–29	–	24–32
Trospectomycin	30 µg	10–16	–	15–20
Trovafloxacin	10 µg	29–36	21–27	29–35
Ulifloxacin (prulifloxacin)[m]	5 µg	32–38	27–33	20–26

Table 4A-1. (Continued)

Antimicrobial Agent	Disk Content	Disk Diffusion QC Ranges, mm *Escherichia coli* ATCC®[b] 25922	*Pseudomonas aeruginosa* ATCC® 27853	*Staphylococcus aureus* ATCC® 25923
Vancomycin	30 µg	–	–	17–21

Abbreviations: AST, antimicrobial susceptibility testing; ATCC®, American Type Culture Collection; ICR, inducible clindamycin resistance; **IQCP, individualized quality control plan;** MHA, Mueller-Hinton agar; QC, quality control.

Footnotes

a. Refer to Table 4A-2 for QC of β-lactam combination agents.

b. ATCC® is a registered trademark of the American Type Culture Collection. Per ATCC® convention, the trademark symbol is used after “BAA” in each catalog number, in conjunction with the registered ATCC® name.

c. *S. aureus* ATCC® 43300 is *mecA* positive and is a supplemental QC strain for testing cefoxitin (acceptable zone ≤ 21 mm).

d. When disk approximation tests are performed with erythromycin and clindamycin, *S. aureus* ATCC® BAA-977™ (containing inducible *erm*[A]-mediated resistance) and *S. aureus* ATCC® BAA-976™ (containing *msr*[A]-mediated macrolide-only efflux) are recommended as supplemental QC strains (eg, for training, competence assessment, or test evaluation). *S. aureus* ATCC® BAA-977™ should demonstrate ICR (ie, a positive D-zone test), whereas *S. aureus* ATCC® BAA-976™ should not demonstrate ICR. *S. aureus* ATCC® 25923 should be used for routine QC (eg, **daily or per IQCP**) of erythromycin and clindamycin disks using standard MHA.

e. QC ranges were established using data from only one disk manufacturer. Disks from other manufacturers were not available at the time of testing.

f. The 200-µg fosfomycin disk contains 50 µg of glucose-6-phosphate.

g. For control ranges of gentamicin 120-µg and streptomycin 300-µg disks, use *Enterococcus faecalis* ATCC® 29212 (gentamicin: 16–23 mm; streptomycin: 14–20 mm).

h. *Klebsiella pneumoniae* ATCC® 700603 is a supplemental QC strain for testing QC of imipenem (25–33 mm) and tebipenem (26–32 mm).

i. Razupenem tested with *S. aureus* ATCC® 25923 can often produce the double or target zone phenomenon. For accurate QC results, use *S. aureus* ATCC® 29213 (no double zones) with acceptable range 33–39 mm.

j. These agents can be affected by excess levels of thymidine and thymine. See CLSI M02[1] for guidance, should a problem with QC occur.

k. Sulfisoxazole can be used to represent any of the currently available sulfonamide preparations.

l. *E. faecalis* ATCC® 29212 is a supplemental QC strain for testing QC of tedizolid (14–21 mm) to assist with reading.

m. Ulifloxacin is the active metabolite of the prodrug prulifloxacin. Only ulifloxacin should be used for AST.

NOTE: Information in boldface type is new or modified since the previous edition.

Table 4A-1. (Continued)

Reference for Table 4A-1

1 CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests*. 14th ed. CLSI standard M02. Clinical and Laboratory Standards Institute; 2024.

Table 4A-2. Disk Diffusion QC Ranges for Nonfastidious Organisms and β-Lactam Combination Agents[a]

Antimicrobial Agent	Disk Content	QC Organisms and Characteristics								
		QC Strains Not Recommended for Routine QC of β-Lactam Combination Agents			QC Strains Recommended for Routine QC of β-Lactam Combination Agents					
		Escherichia coli ATCC®[b] 25922	*Pseudomonas aeruginosa* ATCC® 27853	*Staphylococcus aureus* ATCC® 25923	*Escherichia coli* ATCC® 35218[c,d]	*Klebsiella pneumoniae* ATCC® 700603[c,d,e]	*Escherichia coli* NCTC 13353[c,d]	*Klebsiella pneumoniae* ATCC® BAA-1705™[c,d]	*Klebsiella pneumoniae* ATCC® BAA-2814™	*Acinetobacter baumannii* NCTC 13304[c,d]
		β-Lactamase negative	Inducible AmpC	β-Lactamase negative, *mecA* negative	TEM-1	SHV-18 OXA-2 Mutations in OmpK35 and OmpK37 TEM-1	CTX-M-15 OXA-1	KPC-2 SHV	KPC-3 SHV-11 TEM-1	OXA-27
		Zone Diameter QC Ranges, mm								
Amoxicillin-clavulanate (2:1)	20/10 µg	18–24	–	28–36	17–22	–	–	–	–	–
Ampicillin	10 µg	15–22	–	27–35	6	–	–	–	–	–
Ampicillin-sulbactam (2:1)	10/10 µg	19–24	–	29–37	13–19	–	–	–	–	–
Aztreonam	30 µg	28–36	23–29	–	31–38	10–16	–	–	–	–
Aztreonam-avibactam	30/20 µg	32–38	24–30	–	31–38	26–32[f]	–	–	–	–
Cefepime	30 µg	31–37	25–31	23–29	31–37	23–29	6–15[g]	–	–	6–16[g]
Cefepime-enmetazobactam[f]	30/20 µg	32–38	26–32	–	32–38	26–32	27–33	–	–	–
Cefepime-taniborbactam	30/20 µg	31–37	25–31	–	31–37	24–31	24–30	22–27	–	–
Cefepime-tazobactam	30/20 µg	32–37	27–31	24–30	–	25–30[f]	27–31	–	–	–
Cefepime-zidebactam	30/30 µg	33–40	29–35	–	–	28–34	29–35	–	–	19–25
Cefotaxime	30 µg	29–35	18–22	25–31	–	17–25	–	–	–	–
Cefpodoxime	10 µg	23–28	–	19–25	–	9–16	–	–	–	–
Ceftaroline	30 µg	26–34	–	26–35	–	–	–	–	–	–
Ceftaroline-avibactam	30/15 µg	27–34	17–26	25–34	27–35	21–27[f]	–	–	–	–

Table 4A-2. (Continued)

Antimicrobial Agent	Disk Content	QC Organisms and Characteristics								
		QC Strains Not Recommended for Routine QC of β-Lactam Combination Agents			QC Strains Recommended for Routine QC of β-Lactam Combination Agents					
		Escherichia coli ATCC®[b] 25922	*Pseudomonas aeruginosa* ATCC® 27853	*Staphylococcus aureus* ATCC® 25923	*Escherichia coli* ATCC® 35218[c,d]	*Klebsiella pneumoniae* ATCC® 700603[c,d,e]	*Escherichia coli* NCTC 13353[c,d]	*Klebsiella pneumoniae* ATCC® BAA-1705™[c,d]	*Klebsiella pneumoniae* ATCC® BAA-2814™	*Acinetobacter baumannii* NCTC 13304[c,d]
		β-Lactamase negative	Inducible AmpC	β-Lactamase negative, *mecA* negative	TEM-1	SHV-18 OXA-2 Mutations in OmpK35 and OmpK37 TEM-1	CTX-M-15 OXA-1	KPC-2 SHV	KPC-3 SHV-11 TEM-1	OXA-27
		Zone Diameter QC Ranges, mm								
Ceftazidime	30 µg	25–32	22–29	16–20	–	10–18	–	–	–	–
Ceftazidime-avibactam	30/20 µg	27–35	25–31	16–22	28–35	21–27[f]	–	–	–	–
Ceftibuten	30 µg	–	–	–	–	–	15–23	–	–	–
Ceftibuten-avibactam	**10/4 µg**	**28–36**	**–**	**–**	**–**	**24–30**	**28–34**	**24–30**	**22–28**	**–**
Ceftibuten-ledaborbactam	5/2.5 µg	–	–	–	–	–	24–29	–	–	–
Ceftolozane-tazobactam	30/10 µg	24–32	25–31	10–18	25–31	17–25	–	–	–	–
Ceftriaxone	30 µg	29–35	17–23	22–28	–	16–24	–	–	–	–
Imipenem	10 µg	26–32	20–28	–	–	25–33	–	11–22	6–14	–
Imipenem-relebactam[f]	10/25 µg	27–33	26–31	–	–	26–32	–	23–29	22–28	–
Meropenem[g]	10 µg	28–35	27–33	29–37	–	–	–	11–18[f]	6[f]	–
Meropenem-vaborbactam	20/10 µg	31–37	29–35	32–38	–	29–35	–	21–27	16–20	–
Piperacillin	100 µg	24–30	25–33	–	12–18	–	–	–	–	–
Piperacillin-tazobactam	100/10 µg	24–30	25–33	27–36	24–30	–	–	–	–	–
Sulbactam-durlobactam	10/10 µg	26–32	–	–	–	–	–	–	–	24–30

Table 4A-2. (Continued)

Antimicrobial Agent	Disk Content	QC Organisms and Characteristics								
		QC Strains Not Recommended for Routine QC of β-Lactam Combination Agents			QC Strains Recommended for Routine QC of β-Lactam Combination Agents					
		Escherichia coli ATCC®[b] 25922	*Pseudomonas aeruginosa* ATCC® 27853	*Staphylococcus aureus* ATCC® 25923	*Escherichia coli* ATCC® 35218[c,d]	*Klebsiella pneumoniae* ATCC® 700603[c,d,e]	*Escherichia coli* NCTC 13353[c,d]	*Klebsiella pneumoniae* ATCC® BAA-1705™[c,d]	*Klebsiella pneumoniae* ATCC® BAA-2814™	*Acinetobacter baumannii* NCTC 13304[c,d]
		β-Lactamase negative	Inducible AmpC	β-Lactamase negative, *mecA* negative	TEM-1	SHV-18 OXA-2 Mutations in OmpK35 and OmpK37 TEM-1	CTX-M-15 OXA-1	KPC-2 SHV	KPC-3 SHV-11 TEM-1	OXA-27
		Zone Diameter QC Ranges, mm								
Ticarcillin	75 µg	24–30	21–27	–	6	–	–	–	–	–
Ticarcillin-clavulanate	75/10 µg	24–30	20–28	29–37	21–25	–	–	–	–	–

Abbreviations: ATCC®, American Type Culture Collection; MIC, minimal inhibitory concentration; NCTC, National Collection of Type Cultures; QC, quality control.

QC strain selection codes:

- QC strain is recommended for routine QC; any strain for which the QC range is highlighted in green may be used for this antimicrobial agent.
- Test one of these agents, highlighted in orange, by a disk diffusion or MIC method to confirm the integrity of the respective QC strain.[c,d]

Footnotes

a. Unsupplemented Mueller-Hinton medium. See Table 4A-1 for QC ranges for combination agents from other drug classes.

b. ATCC® is a registered trademark of the American Type Culture Collection. Per ATCC® convention, the trademark symbol is used after "BAA" in each catalog number, in conjunction with the registered ATCC® name.

c. Careful attention to organism maintenance (eg, minimal subcultures) and storage (eg, –60°C or below) is especially important for these QC strains because spontaneous loss of the plasmid encoding the β-lactamase has been documented. If stored at temperatures above –60°C or if repeatedly subcultured, these strains may lose their resistance characteristics and QC results may be outside the acceptable ranges.

Table 4A-2. (Continued)

d. To confirm the integrity of the QC strain, test one of the single β-lactam agents highlighted in orange by either a disk diffusion or MIC test method when the strain is first subcultured from a frozen or lyophilized stock culture. In some cases, only MIC ranges are available to accomplish this confirmation (see Table 5A-2). In-range results for the single agent indicate the QC strain is reliable for QC of β-lactam combination agents. It is not necessary to check the QC strain again with a single agent until a new frozen or lyophilized stock culture is put into use, providing recommendations for handling QC strains as described in CLSI M02[1] and CLSI M07[2] are followed.

e. Strain may demonstrate two colony morphologies: 1) opaque and cream colored and 2) translucent. Both colony morphologies can be used.

f. QC ranges were established using data from only one disk manufacturer. Disks from other manufacturers were not available at the time of testing.

g. If discrete colonies or a haze of growth are present inside the zone of inhibition, measure the colony-free inner zone.

NOTE: Information in boldface type is new or modified since the previous edition.

References for Table 4A-2

[1] CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests*. 14th ed. CLSI standard M02. Clinical and Laboratory Standards Institute; 2024.

[2] CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*. 12th ed. CLSI standard M07. Clinical and Laboratory Standards Institute; 2024.

Table 4B. Disk Diffusion QC Ranges for Fastidious Organisms

Antimicrobial Agent	Disk Content	Disk Diffusion QC Ranges, mm			
		Haemophilus influenzae ATCC®[a] 49247	*Haemophilus influenzae* ATCC® 49766	*Neisseria gonorrhoeae* ATCC® 49226	*Streptococcus pneumoniae* ATCC® 49619[b]
Amoxicillin-clavulanate[c]	20/10 µg	15–23	–	–	–
Ampicillin	10 µg	13–21	–	–	30–36
Ampicillin-sulbactam	10/10 µg	14–22	–	–	–
Azithromycin	15 µg	13–21	–	30–38	19–25
Aztreonam	30 µg	30–38	–	–	–
Cefaclor	30 µg	–	25–31	–	24–32
Cefdinir	5 µg	–	24–31	40–49	26–31
Cefditoren	5 µg	25–34	–	–	27–35
Cefepime	30 µg	25–31	–	37–46	28–35
Cefetamet	10 µg	23–28	–	35–43	–
Cefixime	5 µg	25–33	–	37–45	16–23
Cefmetazole	30 µg	16–21	–	31–36	–
Cefonicid	30 µg	–	30–38	–	–
Cefotaxime	30 µg	31–39	–	38–48	31–39
Cefotetan	30 µg	–	–	30–36	–
Cefoxitin	30 µg	–	–	33–41	–
Cefpodoxime	10 µg	25–31	–	35–43	28–34
Cefprozil	30 µg	–	20–27	–	25–32
Ceftaroline	30 µg	29–39	–	–	31–41
Ceftaroline-avibactam[d]	30/15 µg	30–38	–	–	–
Ceftazidime	30 µg	27–35	–	35–43	–
Ceftazidime-avibactam[d]	30/20 µg	28–34	–	–	23–31
Ceftibuten	30 µg	29–36	–	–	–
Ceftizoxime	30 µg	29–39	–	42–51	28–34
Ceftobiprole[e]	30 µg	28–36	30–38	–	33–39
Ceftolozane-tazobactam[d]	30/10 µg	23–29	–	–	21–29

Table 4B. (Continued)

Antimicrobial Agent	Disk Content	Disk Diffusion QC Ranges, mm			
		Haemophilus influenzae ATCC®[a] 49247	*Haemophilus influenzae* ATCC® 49766	*Neisseria gonorrhoeae* ATCC® 49226	*Streptococcus pneumoniae* ATCC® 49619[b]
Ceftriaxone	30 µg	31–39	–	39–51	30–35
Cefuroxime	30 µg	–	28–36	33–41	–
Cephalothin	30 µg	–	–	–	26–32
Chloramphenicol	30 µg	31–40[f]	–	–	23–27
Ciprofloxacin	5 µg	34–42	–	48–58	–
Clarithromycin	15 µg	11–17[g]	–	–	25–31
Clinafloxacin	5 µg	34–43	–	–	27–34
Clindamycin	2 µg	–	–	–	19–25
Delafloxacin	5 µg	40–51	–	–	28–36[g]
Dirithromycin	15 µg	–	–	–	18–25
Doripenem	10 µg	21–31	–	–	30–38
Doxycycline	30 µg	–	–	–	25–34
Enoxacin	10 µg	–	–	43–51	–
Eravacycline	20 µg	–	–	–	23–30
Ertapenem[e]	10 µg	20–28	27–33	–	28–35
Erythromycin	15 µg	–	–	–	25–30
Faropenem	5 µg	15–22	–	–	27–35
Fleroxacin	5 µg	30–38	–	43–51	–
Fusidic acid	10 µg	–	–	–	9–16
Garenoxacin	5 µg	33–41	–	–	26–33
Gatifloxacin	5 µg	33–41	–	45–56	24–31
Gemifloxacin	5 µg	30–37	–	–	28–34
Gentamicin	10 µg	–	–	15-20	–
Gepotidacin	10 µg	–	–	32–40	22–28
Grepafloxacin	5 µg	32–39	–	44–52	21–28
Iclaprim	5 µg	24–33	–	–	21–29
Imipenem	10 µg	21–29	–	–	–

Table 4B. (Continued)

Antimicrobial Agent	Disk Content	Disk Diffusion QC Ranges, mm			
		Haemophilus influenzae ATCC®[a] 49247	*Haemophilus influenzae* ATCC® 49766	*Neisseria gonorrhoeae* ATCC® 49226	*Streptococcus pneumoniae* ATCC® 49619[b]
Lefamulin	20 µg	22–28	–	–	19–27
Levofloxacin	5 µg	32–40	–	–	20–25
Levonadifloxacin	10 µg	33–41[g]	–	–	24–31[g]
Linezolid	30 µg	–	–	–	25–34
Lomefloxacin	10 µg	33–41	–	45–54	–
Loracarbef	30 µg	–	26–32	–	22–28
Meropenem	10 µg	20–28	–	–	28–35
Moxifloxacin	5 µg	31–39	–	–	25–31
Nafithromycin	15 µg	16–20[g]	–	–	25–31[g]
Nitrofurantoin	300 µg	–	–	–	23–29
Norfloxacin	10 µg	–	–	–	15–21
Ofloxacin	5 µg	31–40	–	43–51	16–21
Omadacycline	30 µg	21–29	–	–	24–32
Oxacillin	1 µg	–	–	–	≤ 12[h]
Penicillin	10 units	–	–	26–34	24–30
Piperacillin-tazobactam	100/10 µg	33–38	–	–	–
Quinupristin-dalfopristin	15 µg	15–21	–	–	19–24
Razupenem	10 µg	24–30	–	–	29–36
Rifampin	5 µg	22–30	–	–	25–30
Solithromycin	15 µg	16–23	–	33–43	25–33
Sparfloxacin	5 µg	32–40	–	43–51	21–27
Spectinomycin	100 µg	–	–	23–29	–
Tedizolid	2 µg	–	–	–	18–25
Telithromycin	15 µg	17–23	–	–	27–33
Tetracycline	30 µg	14–22	–	30–42	27–31
Tigecycline	15 µg	23–31	–	30–40	23–29
Trimethoprim-sulfamethoxazole	1.25/23.75 µg	24–32	–	–	20–28

Table 4B. (Continued)

Antimicrobial Agent	Disk Content	Disk Diffusion QC Ranges, mm			
		Haemophilus influenzae ATCC®[a] 49247	*Haemophilus influenzae* ATCC® 49766	*Neisseria gonorrhoeae* ATCC® 49226	*Streptococcus pneumoniae* ATCC® 49619[b]
Trospectomycin	30 µg	22–29	–	28–35	–
Trovafloxacin	10 µg	32–39	–	42–55	25–32
Vancomycin	30 µg	–	–	–	20–27

Disk Diffusion Testing Conditions for Clinical Isolates and Performance of QC

Organism	*H. influenzae*	*N. gonorrhoeae*	Streptococci and *Neisseria meningitidis*
Medium	HTM MH-F agar	GC agar base and 1% defined growth supplement. The use of a cysteine-free growth supplement is not required for disk diffusion testing.	MHA supplemented with 5% defibrinated sheep blood MH-F agar for *S. pneumoniae* only
Inoculum	Colony suspension	Colony suspension	Colony suspension
Incubation conditions	5% CO_2; 16–18 h; 35°C ± 2°C	5% CO_2; 20–24 h; 36°C ± 1°C (do not exceed 37°C)	5% CO_2; 20–24 h; 35°C ± 2°C

Abbreviations: ATCC®, American Type Culture Collection; CO_2, carbon dioxide; h, hour(s); GC, gonococcus (*Neisseria gonorrhoeae*); HTM, *Haemophilus* test medium; MHA, Mueller-Hinton; MH-F agar, Mueller-Hinton fastidious; QC, quality control.

Footnotes

a. ATCC® is a registered trademark of the American Type Culture Collection.

b. Despite the lack of reliable disk diffusion breakpoints for *S. pneumoniae* with certain β-lactams, *S. pneumoniae* ATCC® 49619 is the strain designated for QC of all disk diffusion tests with all *Streptococcus* spp.

c. When testing on HTM incubated in ambient air, the acceptable QC limits for *Escherichia coli* ATCC® 35218 are 17–22 mm for amoxicillin-clavulanate.

d. QC limits for *E. coli* ATCC® 35218 in HTM: ceftaroline-avibactam 26–34 mm; ceftazidime-avibactam 27–34 mm; ceftolozane-tazobactam 25–31 mm.

e. Either *H. influenzae* ATCC® 49247 or 49766 may be used for routine QC testing with HTM; *H. influenzae* ATCC® 49247 should be used for routine QC testing with MH-F agar.

f. QC limits for *H. influenzae* ATCC® 49247 in MH-F agar: chloramphenicol 28–36 mm.

g. QC ranges for delafloxacin, levonadifloxacin, and nafithromycin, as well as for clarithromycin with MH-F agar, were established using data from only one disk manufacturer. Disks from other manufacturers were not available at the time of testing.

h. Deterioration in oxacillin disk content is best assessed with QC organism *Staphylococcus aureus* ATCC® 25923, with an acceptable zone diameter of 18–24 mm.

Table 4C. Disk Diffusion Reference Guide to QC Frequency to Support Modifications to Antimicrobial Susceptibility Test Systems

This table summarizes the suggested QC frequency when modifications are made to antimicrobial susceptibility test systems (refer to CLSI EP23™[1] **and CLSI M52[2]). Alternative approaches can be used as determined by IQCP. Refer to Appendix I for additional guidance on selection of QC strains and QC testing frequency.**

Test Modification	Recommended QC Frequency			Comments
	1 Day	5 Days	Daily or Per IQCP	
Disks				
Use new shipment or lot number.	X			
Use new manufacturer.	X			
Addition of new antimicrobial agent to existing system.			X	In addition, perform in-house verification studies.
Media (prepared agar plates)				
Use new shipment or lot number.	X			
Use new manufacturer.		X		
Inoculum preparation				
Convert inoculum preparation/ standardization to use of a device that has its own QC protocol.		X		**Example:** Convert from visual adjustment of turbidity to use of a photometric device for which a QC procedure is provided.
Convert inoculum preparation/ standardization to a method that depends on user technique.			X	**Example:** Convert from visual adjustment of turbidity to another method that is not based on a photometric device.
Measuring zones				
Change method of measuring zones.			X	**Example:** Convert from manual zone measurements to automated zone reader. In addition, perform in-house verification studies.
Instrument/software (eg, automated zone reader)				
Software update that affects AST results		X		Monitor all drugs, not just those implicated in software modification.
Repair of instrument that affects AST results	X			Depending on extent of repair (eg, critical component such as the photographic device), additional testing may be appropriate (eg, 5 days).

Abbreviations: AST, antimicrobial susceptibility testing; d, day(s); **IQCP, individualized quality control plan;** QC, quality control.

Table 4C. (Continued)

NOTE 1: QC can be performed before or concurrent with testing patient isolates. Patient results can be reported for that day if QC results are within the acceptable limits.

NOTE 2: Manufacturers of commercial or in-house-prepared tests should follow their own internal procedures and applicable regulations.

NOTE 3: For troubleshooting out-of-range results, refer to CLSI M02[3] and Table 4D. Additional information is available in Appendix C (eg, QC organism characteristics, QC testing recommendations).

NOTE 4: Broth, saline, and/or water used to prepare an inoculum does not need routine QC.

NOTE 5: Information in boldface type is new or modified since the previous edition.

References for Table 4C

[1] CLSI. *Laboratory Quality Control Based on Risk Management*. 2nd ed. CLSI guideline EP23. Clinical and Laboratory Standards Institute; 2023.

[2] **CLSI. *Verification of Commercial Microbial Identification and Antimicrobial Susceptibility Testing Systems*. 1st ed. CLSI guideline M52. Clinical and Laboratory Standards Institute; 2015.**

[3] CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests*. 14th ed. CLSI standard M02. Clinical and Laboratory Standards Institute; 2024.

Table 4D. Disk Diffusion Troubleshooting Guide

This table provides guidance for troubleshooting and corrective action for out-of-range QC, primarily using antimicrobial susceptibility tests with MHA. Refer to CLSI M02[1] for additional information. Out-of-range QC tests are often the result of contamination or the use of an incorrect QC strain; corrective action should first include repeating the test with a pure culture of a freshly subcultured QC strain. If the issue is unresolved, this troubleshooting guide should be consulted regarding additional suggestions for troubleshooting out-of-range QC results and unusual clinical isolate results. In addition, see general corrective action outlined in CLSI M02[1] and notify manufacturers of potential product problems.

General Comment

(1) QC organism maintenance: Avoid repeated subcultures. Retrieve new QC strain from stock (refer to CLSI M02[1]). If using lyophilized strains, follow the maintenance recommendations of the manufacturer.

Antimicrobial Agent	QC Strain	Observation	Probable Cause	Comments/Suggested Actions
β-LACTAMS				
β-Lactam combination agents	*Acinetobacter baumannii* ATCC®[a] 13304 *Escherichia coli* ATCC® 35218 *E. coli* ATCC® 13353 *Klebsiella pneumoniae* ATCC® 700603 *K. pneumoniae* ATCC® BAA-1705™	Zone too large or susceptible for single β-lactam agent; in range for combination β-lactam agent	Spontaneous loss of the plasmid encoding the β-lactamase	Obtain new frozen or lyophilized stock culture. Use other routine QC strains (if available). These strains should be stored at −60°C or below, and frequent subcultures should be avoided. **NOTE:** *K. pneumoniae* BAA-2814™ is stable and does not require QC integrity check.
β-Lactam combination agents	*A. baumannii* ATCC® 13304 *E. coli* ATCC® 35218 *E. coli* ATCC® 13353 *K. pneumoniae* ATCC® 700603 *K. pneumoniae* ATCC® BAA-1705™ *K. pneumoniae* ATCC® BAA-2814™	Zone too small or resistant for both the single β-lactam agent and the combination β-lactam agent	Antimicrobial agent is degrading.	Use alternative lot of test materials. Check storage and package integrity. Imipenem and clavulanate are especially labile.

Table 4D. (Continued)

Antimicrobial Agent	QC Strain	Observation	Probable Cause	Comments/Suggested Actions
β-LACTAMS (Continued)				
Carbenicillin	*Pseudomonas aeruginosa* ATCC® 27853	Zone too small	QC strain develops resistance after repeated subculture.	See general comment (1) on QC strain maintenance.
Cefepime	*A. baumannii* NCTC 13304 *E. coli* NCTC 13353	QC strain integrity test	Discrete colonies may grow within the zone of inhibition when this organism is tested with cefepime 30-µg disk.	If this occurs, measure the colony-free inner zone.
Imipenem	*K. pneumoniae* ATCC® BAA-1705™ *K. pneumoniae* ATCC® BAA-2814™	QC strain integrity test	Discrete colonies may grow within the zone of inhibition when this organism is tested with cefepime 30-µg disk.	If this occurs, measure the colony-free inner zone.
Penicillins	Any	Zone too large	pH of media too low	Acceptable pH range = 7.2–7.4 Avoid CO_2 incubation, which lowers pH.
Penicillins	Any	Zone too small	pH of media too high	Acceptable pH range = 7.2–7.4
β-Lactam group	Any	Zone initially acceptable, but decreases to possibly be out of range over time.	Imipenem, clavulanate, and cefaclor are especially labile. Disks have lost potency.	Use alternative lot of disks. Check storage conditions and package integrity.
NON-β-LACTAMS				
Aminoglycosides Quinolones	Any	Zone too small	pH of media too low	Acceptable pH range = 7.2–7.4 Avoid CO_2 incubation, which lowers pH.
	Any	Zone too large	pH of media too high	Acceptable pH range = 7.2–7.4
Aminoglycosides	*P. aeruginosa* ATCC® 27853	Zone too small	Ca^{2+} and/or Mg^{2+} content too high	Use alternative lot of media.
Aminoglycosides	*P. aeruginosa* ATCC® 27853	Zone too large	Ca^{2+} and/or Mg^{2+} content too low	Use alternative lot of media.
Clindamycin Macrolides	*Staphylococcus aureus* ATCC® 25923	Zone too small	pH of media too low	Acceptable pH range = 7.2–7.4 Avoid CO_2 incubation, which lowers pH.
	S. aureus ATCC® 25923	Zone too large	pH of media too high	Acceptable pH range = 7.2–7.4

Table 4D. (Continued)

Antimicrobial Agent	QC Strain	Observation	Probable Cause	Comments/Suggested Actions
NON-β-LACTAMS (Continued)				
Quinolones	Any	Zone too small	pH of media too low	Acceptable pH range = 7.2–7.4 Avoid CO_2 incubation, which lowers pH.
Quinolones	Any	Zone too large	pH of media too high	Acceptable pH range = 7.2–7.4
Tedizolid	*Enterococcus faecalis* ATCC® 29212	Zone with *Enterococcus* spp. is difficult to read	Light growth on MHA	*E. faecalis* ATCC® 29212 is provided as supplemental QC to assist in personnel training and assessment of proper reading. Measure zone edge where there is a significant decrease in density of growth when using transmitted light as illustrated in the photographs.[b]
Tetracyclines	Any	Zone too large	pH of media too low	Acceptable pH range = 7.2–7.4 Avoid CO_2 incubation, which lowers pH.
Tetracyclines	Any	Zone too small	pH of media too high	Acceptable pH range = 7.2–7.4
Tetracyclines	Any	Zone too small	Ca^{2+} and/or Mg^{2+} content too high	Use alternative lot of media.
Tetracyclines	Any	Zone too large	Ca^{2+} and/or Mg^{2+} content too low	Use alternative lot of media.
Sulfonamides Trimethoprim Trimethoprim-sulfamethoxazole	*E. faecalis* ATCC® 29212	Zone ≤ 20 mm	Media too high in thymidine content	Use alternative lot of media.
ALL AGENTS				
Various	*Streptococcus pneumoniae* ATCC® 49619	Zones too large Lawn of growth scanty	Inoculum source plate too old and contains too many nonviable cells. Plate used to prepare inoculum should be 18–20 h.	Subculture QC strain and repeat QC test or retrieve new QC strain from stock.
Various	Various	Zone too small	Contamination Use of magnification to read zones	Measure zone edge with visible growth detected with unaided eye. Subculture to determine purity and repeat if necessary.

Table 4D. (Continued)

Antimicrobial Agent	QC Strain	Observation	Probable Cause	Comments/Suggested Actions
ALL AGENTS (Continued)				
Various	Any	Many zones too small	Inoculum too heavy Error in inoculum preparation Media depth too thick	Repeat using McFarland 0.5 turbidity standard or standardizing device. Check expiration date and proper storage if using barium sulfate or latex standards. Use agar with depth approximately 4 mm. Recheck alternate lots of MHA.
Various	Any	One or more zones too small or too large	Measurement error Transcription error Random defective disk Disk not pressed firmly against agar	Recheck readings for measurement or transcription errors. Retest. If retest results are out of range and no errors are detected, initiate corrective action.
Various	Various	Zone too large	Did not include lighter growth in zone measurement (eg, double zone, fuzzy zone edge)	Measure zone edge with visible growth detected with unaided eye.
Various	Any	QC results from one strain are out of range, but results from other QC strain(s) is in range with the same antimicrobial agent.	One QC strain may be a better indicator of a QC problem.	Retest this strain to confirm reproducibility of acceptable results. Evaluate with alternative strains with known MICs. Initiate corrective action with problem QC strain/antimicrobial agent(s).
Various	Any	QC results from two strains are out of range with the same antimicrobial agent.	A problem with the disk	Use alternative lot of disks. Check storage conditions and package integrity.
Various	Any	Zones overlap.	Too many disks per plate	Place no more than 12 disks on a 150-mm plate and 5 disks on a 100-mm plate; for some fastidious bacteria that produce large zones, use fewer.

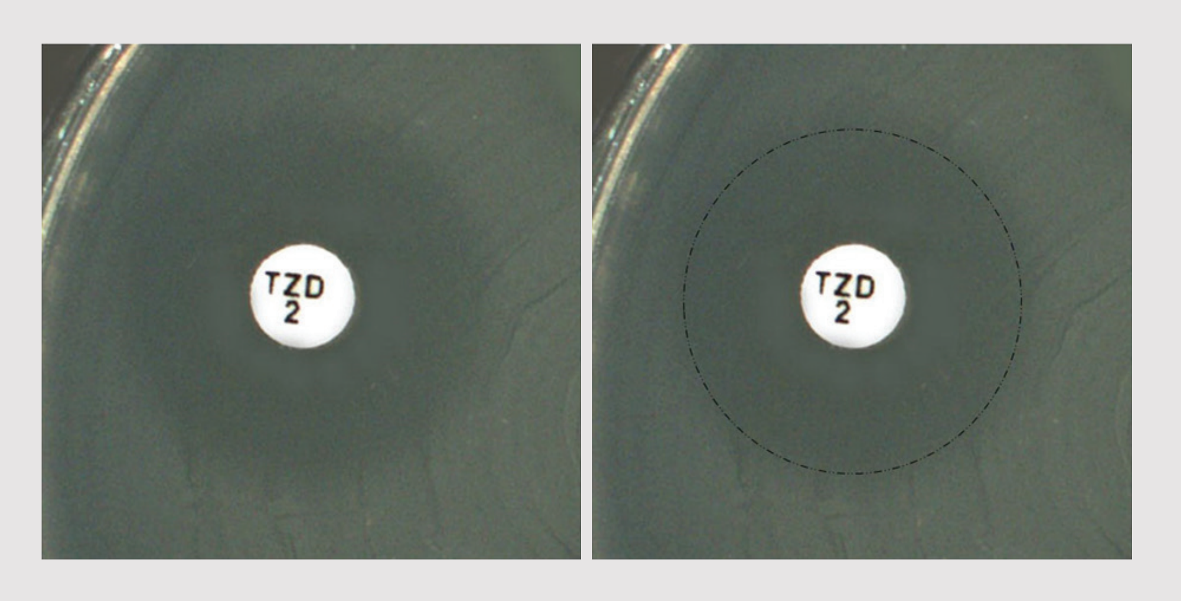
Abbreviations: ATCC®, American Type Culture Collection; CO_2, carbon dioxide; h, hour(s); MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; NCTC, National Collection of Type Cultures; pH, negative logarithm of hydrogen ion concentration; QC, quality control.

Footnotes

a. ATCC® is a trademark of the American Type Culture Collection.

b. Figure 1 shows examples of tedizolid disk diffusion results for *E. faecalis*.

Table 4D. (Continued)



Abbreviation: ATCC®, American Type Culture Collection.

Figure 1. Measuring the Tedizolid Zone for *E. faecalis* ATCC® 29212 When Light Growth Is Observed

Reference for Table 4D

1 CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests*. 14th ed. CLSI standard M02. Clinical and Laboratory Standards Institute; 2024.

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Table 5A-1. MIC QC Ranges for Nonfastidious Organisms and Antimicrobial Agents Excluding β-Lactam Combination Agents[a]

Antimicrobial Agent	MIC QC Ranges, µg/mL			
	Escherichia coli ATCC®[b] 25922	*Pseudomonas aeruginosa* ATCC® 27853	*Staphylococcus aureus* ATCC® 29213	*Enterococcus faecalis* ATCC® 29212
Amikacin	0.5–4	1–4	1–4	64–256
Amikacin-fosfomycin (5:2)[c]	0.25/0.1–2/0.8	1/0.4–8/3.2	0.5/0.2–4/1.6	32/12.8–128/51.2
Amoxicillin	–	–	–	–
Ampicillin	2–8	–	0.5–2	0.5–2
Azithromycin	–	–	0.5–2	–
Azlocillin	8–32	2–8	2–8	1–4
Aztreonam	0.06–0.5	2–8	–	–
Besifloxacin	0.06–0.25	1–4	0.016–0.06	0.06–0.25
Biapenem	0.03–0.12	0.5–2	0.03–0.12	–
Cadazolid	–	–	0.06–0.5	0.06–0.25
Carbenicillin	4–16	16–64	2–8	16–64
Cefaclor	1–4	–	1–4	–
Cefamandole	0.25–1	–	0.25–1	–
Cefazolin	1–4	–	0.25–1	–
Cefdinir	0.12–0.5	–	0.12–0.5	–
Cefditoren	0.12–1	–	0.25–2	–
Cefepime	0.016–0.12	0.5–4	1–4	–
Cefetamet	0.25–1	–	–	–
Cefiderocol[d]	0.06–0.5	0.06–0.5	–	–
Cefixime	0.25–1	–	8–32	–
Cefmetazole	0.25–1	> 32	0.5–2	–
Cefonicid	0.25–1	–	1–4	–
Cefoperazone	0.12–0.5	2–8	1–4	–
Cefotaxime	0.03–0.12	8–32	1–4	–
Cefotetan	0.06–0.25	–	4–16	–
Cefoxitin[e]	2–8	–	1–4	–

Table 5A-1. (Continued)

Antimicrobial Agent	MIC QC Ranges, µg/mL			
	Escherichia coli ATCC®[b] 25922	*Pseudomonas aeruginosa* ATCC® 27853	*Staphylococcus aureus* ATCC® 29213	*Enterococcus faecalis* ATCC® 29212
Cefpodoxime	0.25–1	–	1–8	–
Cefprozil	1–4	–	0.25–1	–
Ceftaroline	0.03–0.12	–	0.12–0.5	0.25–2[f]
Ceftazidime	0.06–0.5	1–4	4–16	–
Ceftibuten[g]	0.12–1	–	–	–
Ceftizoxime	0.03–0.12	16–64	2–8	–
Ceftobiprole	0.03–0.12	1–4	0.12–1	0.06–0.5
Ceftriaxone	0.03–0.12	8–64	1–8	–
Cefuroxime	2–8	–	0.5–2	–
Cephalothin	4–16	–	0.12–0.5	–
Chloramphenicol	2–8	–	2–16	4–16
Cinoxacin	2–8	–	–	–
Ciprofloxacin[h]	0.004–0.016	0.12–1	0.12–0.5	0.25–2
Clarithromycin	–	–	0.12–0.5	–
Clinafloxacin	0.002–0.016	0.06–0.5	0.008–0.06	0.03–0.25
Clindamycin[i]	–	–	0.06–0.25	4–16
Colistin[j,k,l]	–	0.25–2	–	–
Dalbavancin[m]	–	–	0.03–0.12	0.03–0.12
Daptomycin[n]	–	–	0.12–1	1–4
Delafloxacin	0.008–0.03	0.12–0.5	0.001–0.008	0.016–0.12
Dirithromycin	–	–	1–4	–
Doripenem	0.016–0.06	0.12–0.5	0.016–0.06	1–4
Doxycycline	0.5–2	–	0.12–0.5	2–8
Enoxacin	0.06–0.25	2–8	0.5–2	2–16
Eravacycline	0.016–0.12	2–16	0.016–0.12	0.016–0.06
Ertapenem	0.004–0.016	2–8	0.06–0.25	4–16
Erythromycin[i]	–	–	0.25–1	1–4

Table 5A-1. (Continued)

Antimicrobial Agent	MIC QC Ranges, µg/mL			
	Escherichia coli ATCC®[b] 25922	*Pseudomonas aeruginosa* ATCC® 27853	*Staphylococcus aureus* ATCC® 29213	*Enterococcus faecalis* ATCC® 29212
Exebacase[o]	–	–	0.25–2	8–64
Faropenem	0.25–1	–	0.03–0.12	–
Fidaxomicin	–	–	2–16	1–4
Finafloxacin	0.004–0.03	1–8	0.03–0.25	0.25–1
Fleroxacin	0.03–0.12	1–4	0.25–1	2–8
Fosfomycin[p]	0.5–2	2–8	0.5–4	32–128
Fusidic acid	–	–	0.06–0.25	–
Garenoxacin	0.004–0.03	0.5–2	0.004–0.03	0.03–0.25
Gatifloxacin	0.008–0.03	0.5–2	0.03–0.12	0.12–1.0
Gemifloxacin	0.004–0.016	0.25–1	0.008–0.03	0.016–0.12
Gentamicin[q]	0.25–1	0.5–2	0.12–1	4–16
Gepotidacin	1–4	–	0.12–1	1–4
Grepafloxacin	0.004–0.03	0.25–2.0	0.03–0.12	0.12–0.5
Iclaprim	1–4	–	0.06–0.25	0.004–0.03
Imipenem	0.06–0.5	1–4	0.016–0.06	0.5–2
Kanamycin	1–4	–	1–4	16–64
Lefamulin	–	–	0.06–0.25	–
Levofloxacin	0.008–0.06	0.5–4	0.06–0.5	0.25–2
Levonadifloxacin	0.03–0.25	0.5–4	0.008–0.03	–
Linezolid[r]	–	–	1–4	1–4
Lomefloxacin	0.03–0.12	1–4	0.25–2	2–8
Loracarbef	0.5–2	> 8	0.5–2	–
Mecillinam	0.03–0.25[s]	–	–	–
Meropenem	0.008–0.06	0.12–1	0.03–0.12	2–8
Minocycline[h]	0.25–1	–	0.06–0.5	1–4
Moxalactam	0.12–0.5	8–32	4–16	–
Moxifloxacin	0.008–0.06	1–8	0.016–0.12	0.06–0.5

Table 5A-1. (Continued)

Antimicrobial Agent	MIC QC Ranges, µg/mL			
	Escherichia coli ATCC®[b] 25922	*Pseudomonas aeruginosa* ATCC® 27853	*Staphylococcus aureus* ATCC® 29213	*Enterococcus faecalis* ATCC® 29212
Nafcillin	–	–	0.12–0.5	2–8
Nafithromycin	–	–	0.06–0.25	0.016–0.12
Nalidixic acid[h]	1–4	–	–	–
Netilmicin	≤ 0.5–1	0.5–8	≤ 0.25	4–16
Nitrofurantoin	4–16	–	8–32	4–16
Norfloxacin	0.03–0.12	1–4	0.5–2	2–8
Ofloxacin	0.016–0.12	1–8	0.12–1	1–4
Omadacycline[t]	0.25–2	–	0.12–1	0.06–0.5
Oritavancin[m]	–	–	0.016–0.12	0.008–0.03
Oxacillin[e]	–	–	0.12–0.5	8–32
Ozenoxacin	–	–	0.001–0.004	0.016–0.06
Penicillin	–	–	0.25–2	1–4
Pexiganan	2–8	2–16	8–32	16–64
Piperacillin	1–4	1–8	1–4	1–4
Plazomicin	0.25–2	1–4	0.25–2	–
Polymyxin B[u]	0.25–2	0.5–2	–	–
Quinupristin-dalfopristin	–	–	0.25–1	2–8
Razupenem	0.06–0.5	–	0.008–0.03	0.25–1
Rifampin	4–16	16–64	0.004–0.016	0.5–4
Solithromycin	–	–	0.03–0.12	0.016–0.06
Sparfloxacin	0.004–0.016	0.5–2	0.03–0.12	0.12–0.5
Sulfisoxazole[v,w]	8–32	–	32–128	32–128
Sulopenem	0.016–0.06	–	0.016–0.12	2–8
Tebipenem[g]	0.008–0.03	1–8	0.016–0.06	0.25–1
Tedizolid[x]	–	–	0.12–1	0.25–1
Teicoplanin	–	–	0.25–1	0.25–1
Telavancin[m]	–	–	0.03–0.12	0.03–0.12

Table 5A-1. (Continued)

Antimicrobial Agent	MIC QC Ranges, µg/mL			
	Escherichia coli ATCC®[b] 25922	*Pseudomonas aeruginosa* ATCC® 27853	*Staphylococcus aureus* ATCC® 29213	*Enterococcus faecalis* ATCC® 29212
Telithromycin	–	–	0.06–0.25	0.016–0.12
Tetracycline	0.5–2	8–32	0.12–1	8–32
Ticarcillin	4–16	8–32	2–8	16–64
Tigecycline[t]	0.03–0.25	–	0.03–0.25	0.03–0.12
Tobramycin	0.25–1	0.25–1	0.12–1	8–32
Trimethoprim[v]	0.5–2	> 64	1–4	0.12–0.5
Trimethoprim-sulfamethoxazole[v] (1:19)	≤ 0.5/9.5	8/152–32/608	≤ 0.5/9.5	≤ 0.5/9.5
Trospectomycin	8–32	–	2–16	2–8
Trovafloxacin	0.004–0.016	0.25–2	0.008–0.03	0.06–0.25
Ulifloxacin (prulifloxacin)[y]	0.004–0.016	0.12–0.5	–	–
Upleganan[g,z]	0.06–0.25	0.12–0.5	–	–
Vancomycin[aa]	–	–	0.5–2	1–4
Zidebactam	0.06–0.25	1–8	–	–
Zoliflodacin	1–4	–	0.12–0.5	0.25–2
Zosurabalpin[g,bb,cc]	**–**	**–**	**–**	**–**

Abbreviations: AR, antimicrobial resistance; AST, antimicrobial susceptibility testing; ATCC®, American Type Culture Collection; CAMHB, cation-adjusted Mueller-Hinton broth; CAMHB-HSD, cation-adjusted Mueller-Hinton broth supplemented with horse serum (25% v/v) and 0.5 mM DL-dithiothreitol (pH 7.2–7.4); CO_2, carbon dioxide; ICR, inducible clindamycin resistance; LHB, lysed horse blood; MHB, Mueller-Hinton broth; MIC, minimal inhibitory concentration; NCTC, National Collection of Type Cultures; pH, negative logarithm of hydrogen ion concentration; QC, quality control.

Footnotes

a. Refer to Table 5A-2 for QC of β-lactam combination agents.

b. ATCC® is a registered trademark of the American Type Culture Collection. Per ATCC® convention, the trademark symbol is used after "BAA" in each catalog number, in conjunction with the registered ATCC® name.

c. QC ranges reflect MICs obtained when medium is supplemented with 25 µg/mL of glucose-6-phosphate.

d. QC ranges reflect MICs obtained when CAMHB is iron depleted. Chelation is used for iron depletion, which also removes other cations (ie, calcium, magnesium, and zinc). Following this process, cations are added back to concentrations of calcium 20–25 mg/L, magnesium 10–12.5 mg/L, and zinc 0.5–1.0 mg/L.

e. *S. aureus* ATCC® 43300 is *mecA* positive and is a supplemental QC strain for cefoxitin (acceptable MIC ≥ 8 µg/mL) and oxacillin (acceptable MIC ≥ 4 µg/mL).

Table 5A-1. (Continued)

f. Testing this strain with this antimicrobial agent is considered supplemental QC only and is not required as routine user QC testing.

g. MIC ranges were established using broth microdilution only. Equivalency data for agar dilution are not available.

h. QC limits for *E. coli* ATCC® 25922 with ciprofloxacin, nalidixic acid, minocycline, and sulfisoxazole when tested in CAMHB with 2.5% to 5% LHB incubated either in ambient air or 5% CO_2 (when testing *Neisseria meningitidis*) are the same as those listed in this table.

i. When the erythromycin/clindamycin combination well for detecting ICR is used, *S. aureus* ATCC® BAA-977™ (containing inducible *erm*[A]-mediated resistance) and *S. aureus* ATCC® 29213 or *S. aureus* ATCC® BAA-976™ (containing *msr*[A]-mediated macrolide-only efflux) are recommended for QC purposes. *S. aureus* ATCC® BAA-977™ should demonstrate ICR (ie, growth in the well), whereas *S. aureus* ATCC® 29213 and *S. aureus* ATCC® BAA-976™ should not demonstrate ICR (ie, no growth in the well).

j. *P. aeruginosa* ATCC® 27853 is recommended for routine QC. Additional ranges for colistin are also provided as supplemental QC (eg, confirm quality of production lots, validation studies). These supplemental QC strains and ranges for colistin include *E. coli* NCTC 13846 (1–8 µg/mL, bimodal 2–4) and *E. coli* ATCC® BAA-3170™ (formerly AR Bank #0349 *mcr-1*) (1–4 µg/mL, mode 2). Results of 1 µg/mL or 8 µg/mL were infrequent (< 5%) during Tier 2 studies to establish colistin QC ranges. Determine whether MIC results trend at the low or high end of the range (1 µg/mL or 8 µg/mL) (for troubleshooting, see Table 5G).

k. Colistin results are significantly affected by preparation and handling of testing materials, including stock solutions and test medium, as well as by the composition of the testing tube and/or plate (eg, glass, polystyrene, polypropylene). QC results may fall outside the established CLSI QC ranges if methods other than CLSI reference methods described in CLSI M07[1] and CLSI M100 are used.

l. If *P. aeruginosa* ATCC® 27853 frequently tests at 0.25 µg/mL, test *E. coli* NCTC 13846 or *E. coli* ATCC® BAA-3170™.

m. QC ranges reflect MICs obtained when CAMHB is supplemented with 0.002% polysorbate-80.

n. QC ranges reflect MICs obtained when MHB is supplemented with calcium to a final concentration of 50 µg/mL. Agar dilution has not been validated for daptomycin.

o. QC ranges reflect MICs obtained when CAMHB-HSD is incubated in ambient conditions for 16–20 hours or in 5% CO_2 for 20–24 hours. Data based on incubation for 5% CO_2 and 20–24 hours were collected with limited Mueller-Hinton media manufacturers. Agar dilution is not recommended for exebacase testing. *S. aureus* ATCC® 29213 is the routine QC strain; testing may use either incubation protocol described **in Appendix H, section H2,** but MIC end points should be read only **as described for *S. aureus*.** *E. faecalis* ATCC® 29212 is provided for supplemental QC.

p. The approved MIC susceptibility testing method is agar dilution. Agar media should be supplemented with 25 µg/mL of glucose-6-phosphate. Broth dilution should not be performed.

q. For control organisms for gentamicin and streptomycin high-level aminoglycoside tests for enterococci, see Table 3L.

r. QC range for *S. aureus* ATCC® 25923 with linezolid is 1–4 µg/mL; this strain exhibits less trailing, and MIC end points are easier to interpret. *S. aureus* ATCC® 25923 is considered a supplemental QC strain and is not required for routine QC of linezolid MIC tests.

Table 5A-1. (Continued)

s. This test should be performed by agar dilution only.

t. For broth microdilution testing of omadacycline and tigecycline, when MIC panels are prepared, the medium must be prepared fresh on the day of use. The medium must be no more than 12 hours old at the time the panels are made; however, the panels may then be frozen for later use.

u. *E. coli* NCTC 13846 is a supplemental QC strain for polymyxin B with an acceptable range of 1–4 µg/mL, mode 2 µg/mL.

v. Very medium-dependent, especially with enterococci.

w. Sulfisoxazole can be used to represent any of the currently available sulfonamide preparations.

x. QC range for *S. aureus* ATCC® 25923 with tedizolid is 0.12–0.5 µg/mL; this strain exhibits less trailing, and MIC end points are easier to interpret. *S. aureus* ATCC® 25923 is considered a supplemental QC strain and is not required for routine QC of tedizolid MIC tests.

y. Ulifloxacin is the active metabolite of the prodrug prulifloxacin. Only ulifloxacin should be used for AST.

z. *E. coli* NCTC 13846 is a supplemental QC strain for upleganan with an acceptable range of 1–4 µg/mL, mode 2 µg/mL.

aa. For QC organisms for vancomycin screen test for enterococci, see Table 3I.

bb. QC ranges reflect MICs obtained when CAMHB is supplemented with 20% heat-inactivated horse serum.

cc. QC range for *Acinetobacter baumannii* NCTC 13304 with zosurabalpin is 0.016–0.12 µg/mL.

NOTE 1: These MICs were obtained in several referral laboratories by dilution methods. If four or fewer concentrations are tested, QC may be more difficult.

NOTE 2: MIC ranges apply to both broth microdilution and agar dilution unless otherwise specified.

NOTE 3: Information in boldface type is new or modified since the previous edition.

Reference for Table 5A-1

[1] CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically.* 12th ed. CLSI standard M07. Clinical and Laboratory Standards Institute; 2024.

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Table 5A-2. MIC QC Ranges for Nonfastidious Organisms and β-Lactam Combination Agents[a]

Antimicrobial Agent	QC Organisms and Characteristics									
	QC Strains Not Recommended for Routine QC of β-Lactam Combination Agents				QC Strains Recommended for Routine QC of β-Lactam Combination Agents					
	Escherichia coli ATCC®[b] 25922	*Pseudomonas aeruginosa* ATCC® 27853	*Staphylococcus aureus* ATCC® 29213	*Enterococcus faecalis* ATCC® 29212	*Escherichia coli* ATCC® 35218[c,d]	*Klebsiella pneumoniae* ATCC® 700603[c,d,e]	*Escherichia coli* NCTC 13353[c,d]	*Klebsiella pneumoniae* ATCC® BAA-1705™[c,d]	*Klebsiella pneumoniae* ATCC® BAA-2814™	*Acinetobacter baumannii* NCTC 13304[c,d]
	β-Lactamase negative	Inducible AmpC	Weak β-Lactamase negative, *mecA* negative		TEM-1	SHV-18 OXA-2 Mutations in OmpK35 and OmpK37	CTX-M-15 OXA-1	KPC-2 TEM SHV	KPC-3 SHV-11 TEM-1	OXA-27
	MIC QC Ranges, µg/mL									
Amoxicillin	–	–	–	–	–	> 128	–	–	–	–
Amoxicillin-clavulanate (2:1)	2/1–8/4	–	0.12/0.06–0.5/0.25	0.25/0.12–1.0/0.5	4/2–16/8	4/2–16/8	–	–	–	–
Ampicillin	2–8	–	0.5–2	0.5–2	> 32	> 128	–	–	–	–
Ampicillin-sulbactam (2:1)	2/1–8/4	–	–	–	8/4–32/16	8/4–32/16	–	–	–	–
Aztreonam	0.06–0.5	2–8	–	–	0.03–0.12	> 8	–	–	> 128	–
Aztreonam-avibactam	0.03/4–0.12/4	2/4–8/4	–	–	0.016/4–0.06/4	0.06/4–0.5/4	–	–	–	–
Aztreonam-nacubactam (1:1)	0.06/0.06–0.25/0.25	2/2–8/8	–	–	–	0.5/0.5–2/2	–	–	0.5/0.5–2/2	–
Cefepime	0.016–0.12	0.5–4	1–4	–	0.008–0.06	0.5–2	≥ 64	–	> 32	16–128
Cefepime-enmetazobactam	0.03/8–0.12/8	0.5/8–2/8	–	–	0.008/8–0.06/8	0.12/8–0.5/8	0.03/8–0.12/8	–	–	–
Cefepime-nacubactam (1:1)	0.016/0.016–0.12/0.12	0.5/0.5–2/2	–	–	–	0.12/0.12–0.5/0.5	–	–	0.5/0.5–2/2	–
Cefepime-taniborbactam	0.03/4–0.12/4	0.5/4–4/4	–	–	0.016/4–0.06/4	0.12/4–0.5/4	0.12/4–1/4	0.12/4–0.5/4	–	–

Table 5A-2. (Continued)

Antimicrobial Agent	QC Organisms and Characteristics									
	QC Strains Not Recommended for Routine QC of β-Lactam Combination Agents				QC Strains Recommended for Routine QC of β-Lactam Combination Agents					
	Escherichia coli ATCC®[b] 25922	*Pseudomonas aeruginosa* ATCC® 27853	*Staphylococcus aureus* ATCC® 29213	*Enterococcus faecalis* ATCC® 29212	*Escherichia coli* ATCC® 35218[c,d]	*Klebsiella pneumoniae* ATCC® 700603[c,d,e]	*Escherichia coli* NCTC 13353[c,d]	*Klebsiella pneumoniae* ATCC® BAA-1705™[c,d]	*Klebsiella pneumoniae* ATCC® BAA-2814™	*Acinetobacter baumannii* NCTC 13304[c,d]
	β-Lactamase negative	Inducible AmpC	Weak β-Lactamase negative, *mecA* negative		TEM-1	SHV-18 OXA-2 Mutations in OmpK35 and OmpK37	CTX-M-15 OXA-1	KPC-2 TEM SHV	KPC-3 SHV-11 TEM-1	OXA-27
	MIC QC Ranges, µg/mL									
Cefepime-tazobactam	0.03/8–0.12/8	0.5/8–4/8	1/8–4/8	–	–	0.12/8–0.5/8	0.06/8–0.25/8	–	–	–
Cefepime-zidebactam (1:1)	0.016–0.06	0.5–2	–	–	–	0.06–0.25	0.06–0.5	–	–	4–16
Zidebactam[f]	0.06–0.25	1–8	–	–	–	–	0.06–0.5	–	–	≥ 128
Cefotaxime	0.03–0.12	8–32	1–4	–	–	–	–	–	–	–
Cefpodoxime	0.25–1	–	1–8	–	0.12–0.5	4–32	32–128	–	–	–
Ceftaroline	0.03–0.12	–	0.12–0.5	0.25–2	–	2–8	–	–	–	–
Ceftaroline-avibactam	0.03/4–0.12/4	–	0.12/4–0.5/4	–	0.016/4–0.06/4	0.25/4–1/4	–	–	–	–
Ceftazidime	0.06–0.5	1–4	4–16	–	–	16–64	–	–	–	–
Ceftazidime-avibactam[g]	0.06/4–0.5/4	0.5/4–4/4	4/4–16/4	–	0.03/4–0.12/4	0.25/4–2/4	0.12/4–0.5/4	0.25/4–2/4	1/4–4/4	–
Ceftibuten[g]	0.12–1	–	–	–	–	0.25–1	16–64	4–32	8–32	–
Ceftibuten-avibactam[g]	0.016/4–0.12/4	–	–	–	–	0.06/4–0.25/4	0.03/4–0.12/4	0.03/4–0.25/4	0.12/4–0.5/4	–
Ceftibuten-ledaborbactam	–	–	–	–	–	–	0.03/4–0.25/4	0.12/4–0.5/4	0.5/4–2/4	–
Ceftibuten-xeruborbactam[g]	**–**	**–**	**–**	**–**	**–**	**0.016/4–0.12/4**	**–**	**0.03/4–0.25/4**	**0.12/4–0.5/4**	**–**

Table 5A-2. (Continued)

Antimicrobial Agent	QC Organisms and Characteristics									
	QC Strains Not Recommended for Routine QC of β-Lactam Combination Agents				QC Strains Recommended for Routine QC of β-Lactam Combination Agents					
	Escherichia coli ATCC®[b] 25922	*Pseudomonas aeruginosa* ATCC® 27853	*Staphylococcus aureus* ATCC® 29213	*Enterococcus faecalis* ATCC® 29212	*Escherichia coli* ATCC® 35218[c,d]	*Klebsiella pneumoniae* ATCC® 700603[c,d,e]	*Escherichia coli* NCTC 13353[c,d]	*Klebsiella pneumoniae* ATCC® BAA-1705™[c,d]	*Klebsiella pneumoniae* ATCC® BAA-2814™	*Acinetobacter baumannii* NCTC 13304[c,d]
	β-Lactamase negative	Inducible AmpC	Weak β-Lactamase negative, *mecA* negative		TEM-1	SHV-18 OXA-2 Mutations in OmpK35 and OmpK37	CTX-M-15 OXA-1	KPC-2 TEM SHV	KPC-3 SHV-11 TEM-1	OXA-27
	MIC QC Ranges, µg/mL									
Ceftolozane-tazobactam	0.12/4–0.5/4	0.25/4–1/4	16/4–64/4	–	0.06/4–0.25/4	0.5/4–2/4	–	–	–	–
Ceftriaxone	0.03–0.12	8–64	1–8	–	–	–	–	–	–	–
Durlobactam	0.12–0.5	–	–	–	–	–	–	–	–	32–128
Imipenem	0.06–0.5	1–4	0.016–0.06	0.5–2	–	0.06–0.5	–	4–16	16–64	–
Imipenem-funobactam	0.06/8–0.25/8	0.25/8–1/8	–	–	–	0.06/8–0.25/8	–	0.06/8–0.25/8	–	–
Imipenem-relebactam	0.06/4–0.5/4	0.25/4–1/4	0.008/4–0.03/4	0.5/4–2/4	0.06/4–0.25/4	0.06/4–0.5/4	–	0.03/4–0.25/4	0.06/4–0.5/4	–
Meropenem[h]	0.008–0.06	0.12–1	0.03–0.12	2–8	0.008–0.06	–	0.016–0.06	8–64	32–256	32–128
Meropenem-nacubactam (1:1)	0.016/0.016–0.06/0.06	0.12/0.12–1/1	–	–	–	–	–	–	0.5/0.5–2/2	–
Meropenem-vaborbactam[e]	0.008/8–0.06/8	0.12/8–1/8	0.03/8–0.12/8	–	0.008/8–0.06/8	0.016/8–0.06/8	–	0.008/8–0.06/8	0.12/8–0.5/8	–
Meropenem-xeruborbactam[i]	–	0.06/8–0.5/8	–	–	–	–	–	–	0.015/8–0.06/8	–
Nacubactam[f]	0.5–4	64–256	–	–	–	–	–	–	0.5–4	–
Piperacillin	1–4	1–8	1–4	1–4	> 64	–	–	–	–	–
Piperacillin-tazobactam	1/4–8/4	1/4–8/4	0.25/4–2/4	1/4–4/4	0.5/4–2/4	8/4–32/4	–	–	–	–
Sulbactam	16–64	–	–	–	–	32–128	–	–	–	16–64

Table 5A-2. (Continued)

Antimicrobial Agent	QC Organisms and Characteristics									
	QC Strains Not Recommended for Routine QC of β-Lactam Combination Agents				QC Strains Recommended for Routine QC of β-Lactam Combination Agents					
	Escherichia coli ATCC®[b] 25922	*Pseudomonas aeruginosa* ATCC® 27853	*Staphylococcus aureus,* ATCC® 29213	*Enterococcus faecalis* ATCC® 29212	*Escherichia coli* ATCC® 35218[c,d]	*Klebsiella pneumoniae* ATCC® 700603[c,d,e]	*Escherichia coli* NCTC 13353[c,d]	*Klebsiella pneumoniae* ATCC® BAA-1705™[c,d]	*Klebsiella pneumoniae* ATCC® BAA-2814™	*Acinetobacter baumannii* NCTC 13304[c,d]
	β-Lactamase negative	Inducible AmpC	Weak β-Lactamase negative, *mecA* negative		TEM-1	SHV-18 OXA-2 Mutations in OmpK35 and OmpK37	CTX-M-15 OXA-1	KPC-2 TEM SHV	KPC-3 SHV-11 TEM-1	OXA-27
	MIC QC Ranges, µg/mL									
Sulbactam-durlobactam	–	–	–	–	–	–	–	–	–	0.5/4–2/4
Ticarcillin	4–16	8–32	2–8	16–64	> 128	> 256	–	–	–	–
Ticarcillin-clavulanate	4/2–16/2	8/2–32/2	0.5/2–2/2	16/2–64/2	8/2–32/2	32/2–128/2	–	–	–	–

Abbreviations: ATCC®, American Type Culture Collection; I, intermediate; MIC, minimal inhibitory concentration; NCTC, National Collection of Type Cultures; QC, quality control; R, resistant; S, susceptible.

QC strain selection codes:

- QC strain is recommended for routine QC; any strain for which the QC range is highlighted in green may be used for this antimicrobial agent.
- Test one of these agents, highlighted in orange, by a disk diffusion or MIC method to confirm the integrity of the respective QC strain.[c,d]

Footnotes

a. Unsupplemented Mueller-Hinton medium (cation-adjusted if broth). See Table 5A-1 for QC ranges for combination agents from other drug classes.

b. ATCC® is a registered trademark of the American Type Culture Collection. Per ATCC® convention, the trademark symbol is used after "BAA" in each catalog number, in conjunction with the registered ATCC® name.

c. Careful attention to organism maintenance (eg, minimal subcultures) and storage (eg, –60°C or below) is especially important for these QC strains because spontaneous loss of the plasmid encoding the β-lactamase has been documented. If stored at temperatures above –60°C or if repeatedly subcultured, these strains may lose their resistance characteristics and QC results may be outside the acceptable ranges.

Table 5A-2. (Continued)

d. To confirm the integrity of the QC strain, test one of the single β-lactam agents highlighted in orange by either a disk diffusion or MIC test method when the strain is first subcultured from a frozen or lyophilized stock culture. In-range results for the single agent indicate the QC strain is reliable for QC of β-lactam combination agents. It is not necessary to check the QC strain again with a single agent until a new frozen or lyophilized stock culture is put into use, providing recommendations for handling QC strains as described in CLSI M02[1] and CLSI M07[2] are followed. If the highest concentration tested on a panel is lower than the QC range listed for the particular antimicrobial agent and the MIC result obtained for the QC strain is interpreted as resistant, the QC strain can be considered reliable for QC of β-lactam combination agents (eg, ampicillin panel concentrations 1–16 µg/mL; ampicillin Enterobacterales breakpoints [µg/mL]: ≤ 8 [S], 16 [I], ≥ 32 [R]; MIC of > 16 µg/mL [R] would be acceptable for *K. pneumoniae* ATCC® 700603).

e. Strain may demonstrate two colony morphologies: 1) opaque and cream colored and 2) translucent. Both colony morphologies can be used.

f. Not tested as a single agent routinely.

g. MIC ranges were established using broth microdilution only. Equivalency data for agar dilution are not available.

h. Additional QC strain and range for meropenem include *P. aeruginosa* ATCC® BAA-3197™ (formerly *P. aeruginosa* PA5257) (128-1024 µg/mL) to be used as integrity check strain.

i. Additional QC strain and range for *P. aeruginosa* ATCC® BAA-3197™ (formerly *P. aeruginosa* PA5257) (1/8-4/8 µg/mL) provided as supplemental QC strain.

NOTE 1: MIC ranges apply to both broth microdilution and agar dilution unless otherwise specified.

NOTE 2: Information in boldface type is new or modified since the previous edition.

References for Table 5A-2

[1] CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests*. 14th ed. CLSI standard M02. Clinical and Laboratory Standards Institute; 2024.

[2] CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*. 12th ed. CLSI standard M07. Clinical and Laboratory Standards Institute; 2024.

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Table 5B. MIC QC Ranges for Fastidious Organisms (Broth Dilution Methods)

Antimicrobial Agent	**MIC QC Ranges, µg/mL**		
	Haemophilus influenzae **ATCC®[a] 49247**	***Haemophilus influenzae*** **ATCC® 49766**	***Streptococcus pneumoniae*** **ATCC® 49619**
Amikacin-fosfomycin (5:2)[b]	0.5/0.2–4/1.6	–	8/3.2–64/25.6
Amoxicillin	–	–	0.03–0.12
Amoxicillin-clavulanate (2:1)[c]	2/1–16/8	–	0.03/0.016–0.12/0.06
Ampicillin	2–8	–	0.06–0.25
Ampicillin-sulbactam (2:1)	2/1–8/4	–	–
Azithromycin	1–4	–	0.06–0.25
Aztreonam	0.12–0.5	–	–
Besifloxacin	0.016–0.06	–	0.03–0.12
Cefaclor	–	1–4	1–4
Cefamandole	–	0.25–1	–
Cefdinir	–	0.12–0.5	0.03–0.25
Cefditoren	0.06–0.25	–	0.016–0.12
Cefepime	0.5–2	–	0.03–0.25
Cefepime-tazobactam	0.5/8–2/8	–	0.03/8–0.12/8
Cefetamet	0.5–2	–	0.5–2
Cefixime	0.12–1	–	–
Cefmetazole	2–16	–	–
Cefonicid	–	0.06–0.25	–
Cefotaxime	0.12–0.5	–	0.03–0.12
Cefotetan	–	–	–
Cefoxitin	–	–	–
Cefpirome	0.25–1	–	–
Cefpodoxime	0.25–1	–	0.03–0.12
Cefprozil	–	1–4	0.25–1
Ceftaroline	0.03–0.12	–	0.008–0.03
Ceftaroline-avibactam	0.016/4–0.12/4	–	–

Table 5B. (Continued)

Antimicrobial Agent	MIC QC Ranges, µg/mL		
	Haemophilus influenzae ATCC®[a] 49247	*Haemophilus influenzae* ATCC® 49766	*Streptococcus pneumoniae* ATCC® 49619
Ceftazidime	0.12–1	–	–
Ceftazidime-avibactam[d,e]	0.06/4–0.5/4	0.016/4–0.06/4	0.25/4–2/4
Ceftibuten[d]	0.25–1	–	–
Ceftizoxime	0.06–0.5	–	0.12–0.5
Ceftobiprole[f]	0.12–1	0.016–0.06	0.004–0.03
Ceftolozane-tazobactam	0.5/4–2/4	–	0.25/4–1/4
Ceftriaxone	0.06–0.25	–	0.03–0.12
Cefuroxime	–	0.25–1	0.25–1
Cephalothin	–	–	0.5–2
Chloramphenicol	0.25–1	–	2–8
Ciprofloxacin[g]	0.004–0.03	–	–
Clarithromycin	4–16	–	0.03–0.12
Clinafloxacin	0.001–0.008	–	0.03–0.12
Clindamycin	–	–	0.03–0.12
Dalbavancin[h]	–	–	0.008–0.03
Daptomycin[i]	–	–	0.06–0.5
Delafloxacin	0.00025–0.001	–	0.004–0.016
Dirithromycin	8–32	–	0.06–0.25
Doripenem	–	0.06–0.25	0.03–0.12
Doxycycline	–	–	0.016–0.12
Enoxacin	–	–	–
Eravacycline	0.06–0.5	–	0.004–0.03
Ertapenem	–	0.016–0.06	0.03–0.25
Erythromycin	–	–	0.03–0.12
Faropenem	–	0.12–0.5	0.03–0.25
Finafloxacin	–	0.002–0.008	0.25–1
Fleroxacin	0.03–0.12	–	–

Table 5B. (Continued)

Antimicrobial Agent	MIC QC Ranges, µg/mL *Haemophilus influenzae* ATCC®[a] 49247	MIC QC Ranges, µg/mL *Haemophilus influenzae* ATCC® 49766	MIC QC Ranges, µg/mL *Streptococcus pneumoniae* ATCC® 49619
Fusidic acid	–	–	4–32
Garenoxacin	0.002–0.008	–	0.016–0.06
Gatifloxacin	0.004–0.03	–	0.12–0.5
Gemifloxacin	0.002–0.008	–	0.008–0.03
Gentamicin	–	–	–
Gepotidacin	0.25–1	–	0.06–0.25
Grepafloxacin	0.002–0.016	–	0.06–0.5
Iclaprim	0.12–1	–	0.03–0.12
Imipenem	–	0.25–1	0.03–0.12
Imipenem-relebactam	–	0.25/4–1/4	0.016/4–0.12/4
Lefamulin	0.5–2	–	0.06–0.5
Levofloxacin	0.008–0.03	–	0.5–2
Levonadifloxacin	0.008–0.06	–	0.12–0.5
Linezolid	–	–	0.25–2
Lomefloxacin	0.03–0.12	–	–
Loracarbef	–	0.5–2	2–8
Meropenem	–	0.03–0.12	0.03–0.25
Metronidazole	–	–	–
Minocycline[g]	–	–	–
Moxifloxacin	0.008–0.03	–	0.06–0.25
Nafithromycin	2–8	–	0.008–0.03
Nalidixic acid[g]	–	–	–
Nitrofurantoin	–	–	4–16
Norfloxacin	–	–	2–8
Ofloxacin	0.016–0.06	–	1–4
Omadacycline[j]	0.5–2	–	0.016–0.12
Oritavancin[h]	–	–	0.001–0.004

Table 5B. (Continued)

Antimicrobial Agent	MIC QC Ranges, µg/mL		
	Haemophilus influenzae ATCC®[a] 49247	*Haemophilus influenzae* ATCC® 49766	*Streptococcus pneumoniae* ATCC® 49619
Ozenoxacin	–	–	0.008–0.06
Penicillin	–	–	0.25–1
Pexiganan	8–32	–	16–64
Piperacillin-tazobactam	0.06/4–0.5/4	–	–
Quinupristin-dalfopristin	2–8	–	0.25–1
Razupenem	–	0.008–0.03	0.008–0.06
Rifampin	0.25–1	–	0.016–0.06
Solithromycin	1–4	–	0.004–0.016
Sparfloxacin	0.004–0.016	–	0.12–0.5
Spectinomycin	–	–	–
Sulopenem	–	0.06–0.25	0.03–0.12
Tebipenem[d]	–	0.06-0.25[k]	0.004-0.03
Tedizolid	–	–	0.12–0.5
Telavancin[h]	–	–	0.004–0.016
Telithromycin	1–4	–	0.004–0.03
Tetracycline	4–32	–	0.06–0.5
Tigecycline[j]	0.06–0.5	–	0.016–0.12
Trimethoprim-sulfamethoxazole (1:19)	0.03/0.59–0.25/4.75	–	0.12/2.4–1/19
Trospectomycin	0.5–2	–	1–4
Trovafloxacin	0.004–0.016	–	0.06–0.25
Vancomycin	–	–	0.12–0.5
Zoliflodacin	0.12–1	–	0.12–0.5

Table 5B. (Continued)

MIC Testing Conditions for Clinical Isolates and Performance of QC

Organism	*H. influenzae*	*S. pneumoniae* and streptococci	*Neisseria meningitidis*
Medium	Broth dilution: HTM broth or MH-F broth	Broth dilution: CAMHB with LHB (2.5% to 5% v/v)	Broth dilution: CAMHB with LHB (2.5% to 5% v/v)
Inoculum	Colony suspension	Colony suspension	Colony suspension
Incubation conditions	Ambient air; 20–24 h; 35°C ± 2°C	Ambient air; 20–24 h; 35°C ± 2°C	5% CO_2; 20–24 h; 35°C ± 2°C (for QC with *S. pneumoniae* ATCC® 49619, 5% CO_2 or ambient air, except for azithromycin, ambient air only)

Abbreviations: ATCC®, American Type Culture Collection; CAMHB, cation-adjusted Mueller-Hinton broth; h, hour(s); HTM, *Haemophilus* test medium; LHB, lysed horse blood; MHB, Mueller-Hinton; MH-F, Mueller-Hinton fastidious; MIC, minimal inhibitory concentration; QC, quality control.

Footnotes

a. ATCC® is a registered trademark of the American Type Culture Collection.

b. QC ranges reflect MICs obtained when medium is supplemented with 25 µg/mL of glucose-6-phosphate.

c. QC limits for *Escherichia coli* ATCC® 35218 when tested on HTM are 4/2–16/8 µg/mL for amoxicillin-clavulanate and ≥ 256 µg/mL for amoxicillin; testing amoxicillin may help to determine if the isolate has maintained its ability to produce β-lactamase.

d. MIC ranges were established using broth microdilution only. Equivalency data for agar dilution are not available.

e. QC limits for *Klebsiella pneumoniae* ATCC® 700603 with ceftazidime-avibactam when testing in HTM are 0.25/4–1/4 µg/mL. *K. pneumoniae* ATCC® 700603 should be tested against ceftazidime-avibactam and ceftazidime alone to confirm the activity of avibactam in the combination and to ensure that the plasmid encoding the β-lactamase has not been lost in this strain. The acceptable range for ceftazidime alone is > 16 µg/mL.

f. Either *H. influenzae* ATCC® 49247 or 49766 may be used for routine QC testing.

g. QC limits for *E. coli* ATCC® 25922 with ciprofloxacin, nalidixic acid, minocycline, and sulfisoxazole when tested in CAMHB with 2.5% to 5% LHB incubated either in ambient air or 5% CO_2 (when testing *N. meningitidis*) are the same as those listed in Table 5A-1.

h. QC ranges reflect MICs obtained when CAMHB is supplemented with 0.002% polysorbate-80.

i. QC ranges reflect MICs obtained when MHB is supplemented with calcium to a final concentration of 50 µg/mL. Agar dilution has not been validated for daptomycin.

j. For broth microdilution testing of omadacycline and tigecycline, when MIC panels are prepared, the medium must be prepared fresh on the day of use. The medium must be no more than 12 hours old at the time the panels are made; however, the panels may then be frozen for later use.

Table 5B. (Continued)

k. QC ranges were established with a limited number of media manufacturers.

NOTE 1: For four-dilution ranges, results at the extremes of the acceptable ranges should be suspect. Verify validity with data from other QC strains.

NOTE 2: MIC ranges apply to both broth microdilution and agar dilution unless otherwise specified.

Table 5C. MIC QC Ranges for *Neisseria gonorrhoeae* (Agar Dilution Method)

Antimicrobial Agent	MIC QC Ranges, µg/mL
	N. gonorrhoeae ATCC®[a] 49226
Azithromycin	0.25–1
Cefdinir	0.008–0.03
Cefepime	0.016–0.06
Cefetamet	0.016–0.25
Cefixime	0.004–0.03
Cefmetazole	0.5–2
Cefotaxime	0.016–0.06
Cefotetan	0.5–2
Cefoxitin	0.5–2
Cefpodoxime	0.03–0.12
Ceftazidime	0.03–0.12
Ceftizoxime	0.008–0.03
Ceftriaxone	0.004–0.016
Cefuroxime	0.25–1
Ciprofloxacin	0.001–0.008
Enoxacin	0.016–0.06
Fleroxacin	0.008–0.03
Gatifloxacin	0.002–0.016
Gentamicin	4–16
Gepotidacin	0.25–1
Grepafloxacin	0.004–0.03
Lomefloxacin	0.008–0.03
Moxifloxacin	0.008–0.03
Ofloxacin	0.004–0.016
Penicillin	0.25–1
Solithromycin	0.03–0.25
Sparfloxacin	0.004–0.016

Table 5C. (Continued)

Antimicrobial Agent	MIC QC Ranges, µg/mL
	N. gonorrhoeae **ATCC®[a] 49226**
Spectinomycin	8–32
Tetracycline	0.25–1
Trospectomycin	1–4
Trovafloxacin	0.004–0.016
Zoliflodacin	0.06–0.5

Testing Conditions for Clinical Isolates and Performance of QC

Organism	*N. gonorrhoeae*
Medium	Agar dilution: GC agar base and 1% defined growth supplement. The use of a cysteine-free supplement is necessary for agar dilution tests with carbapenems and clavulanate. Cysteine-containing defined growth supplements do not significantly alter dilution test results with other drugs.
Inoculum	Colony suspension, equivalent to a 0.5 McFarland standard
Incubation conditions	36°C ± 1°C (do not exceed 37°C); 5% CO_2; 20–24 h

Abbreviations: ATCC®, American Type Culture Collection; h, hour(s): GC, gonococcus (*Neisseria gonorrhoaea*); MIC, minimal inhibitory concentration; QC, quality control.

Footnote

a. ATCC® is a registered trademark of the American Type Culture Collection.

Table 5D. MIC QC Ranges for Anaerobes (Agar Dilution Method)

Antimicrobial Agent	MIC QC Ranges, µg/mL			
	Bacteroides fragilis ATCC®[a] 25285	*Bacteroides thetaiotaomicron* ATCC® 29741	*Clostridioides* (formerly *Clostridium*) *difficile* ATCC® 700057	*Eggerthella lenta* (formerly *Eubacterium lentum*) ATCC® 43055[b]
Amoxicillin-clavulanate (2:1)	0.25/0.125–1/0.5	0.5/0.25–2/1	0.25/0.125–1/0.5	–
Ampicillin	16–64	16–64	1–4	–
Ampicillin-sulbactam (2:1)	0.5/0.25–2/1	0.5/0.25–2/1	0.5/0.25–4/2	0.25/0.125–2/1
Cadazolid	–	–	0.12–0.5	–
Cefmetazole	8–32	32–128	–	4–16
Cefoperazone	32–128	32–128	–	32–128
Cefotaxime	8–32	16–64	–	64–256
Cefotetan	4–16	32–128	–	32–128
Cefoxitin	4–16	8–32	–	4–16
Ceftaroline	4–32	16–128	2–16	8–32
Ceftaroline-avibactam	0.12/4–0.5/4	4/4–16/4	0.5/4–4/4	4/4–16/4
Ceftizoxime	–	4–16	–	16–64
Ceftolozane-tazobactam	0.12/4–1/4	16/4–128/4	–	–
Ceftriaxone	32–128	64–256	–	–
Chloramphenicol	2–8	4–16	–	–
Clinafloxacin	0.03–0.125	0.06–0.5	–	0.03–0.125
Clindamycin	0.5–2	2–8	2–8	0.06–0.25
Doripenem	–	–	0.5–4	–
Eravacycline	0.06–0.25	0.12–1	0.06–0.25	–
Ertapenem	0.06–0.25	0.25–1	–	0.5–2
Faropenem	0.03–0.25	0.12–1	–	1–4
Fidaxomicin	–	–	0.03–0.25	–
Finafloxacin	0.12–0.5	1–4	1–4	0.12–0.5
Garenoxacin	0.06–0.5	0.25–1	0.5–2	1–4
Imipenem	0.03–0.125	0.125–0.5	–	0.125–0.5
Imipenem-relebactam	0.03/4–0.25/4	0.06/4–0.5/4	–	0.12/4–1/4

Table 5D. (Continued)

Antimicrobial Agent	MIC QC Ranges, µg/mL			
	Bacteroides fragilis ATCC®[a] 25285	*Bacteroides thetaiotaomicron* ATCC® 29741	*Clostridioides* (formerly *Clostridium*) *difficile* ATCC® 700057	*Eggerthella lenta* (formerly *Eubacterium lentum*) ATCC® 43055[b]
Linezolid	2–8	2–8	1–4	0.5–2
Meropenem	0.03–0.25	0.125–0.5	0.5–4	0.125–1
Metronidazole	0.25–1	0.5–2	0.125–0.5	–
Moxifloxacin	0.125–0.5	1–4	1–4	0.125–0.5
Nitazoxanide	–	–	0.06–0.5	–
Omadacycline	0.25–2	0.5–4	0.25–2	0.25–2
Penicillin	8–32	8–32	1–4	–
Piperacillin	2–8	8–32	4–16	8–32
Piperacillin-tazobactam	0.125/4–0.5/4	4/4–16/4	4/4–16/4	4/4–16/4
Ramoplanin	–	–	0.125–0.5	–
Razupenem	0.016–0.12	0.06–0.25	0.06–0.25	0.06–0.5
Ridinilazole	–	–	0.06–0.25	–
Rifaximin	–	–	0.004–0.016	–
Secnidazole	0.25–1	0.5–2	0.06–0.5	0.25–2
Sulopenem	–	0.06–0.5	1–4	0.5–2
Surotomycin[c]	–	–	0.12–1	2–8
Tebipenem	0.03-0.25	0.12-0.5	0.5-2	0.06-0.25
Tetracycline	0.125–0.5	8–32	–	–
Ticarcillin	16–64	16–64	16–64	16–64
Ticarcillin-clavulanate	–	0.5/2–2/2	16/2–64/2	16/2–64/2
Tigecycline	0.12–1	0.5–2	0.125–1	0.06–0.5
Tinidazole	–	–	0.125–0.5	–
Tizoxanide	–	–	0.06–0.5	–
Vancomycin	–	–	0.5–4	–

Abbreviations: ATCC®, American Type Culture Collection; MIC, minimal inhibitory concentration; QC, quality control.

Table 5D. (Continued)

Footnotes

a. ATCC® is a registered trademark of the American Type Culture Collection.

b. MIC variability with some agents has been reported with *E. lenta* ATCC® 43055; therefore, QC ranges have not been established for all antimicrobial agents with this organism.

c. QC ranges reflect MICs obtained when media are supplemented with calcium to a final concentration of 50 µg/mL.

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Table 5E. MIC QC Ranges for Anaerobes (Broth Microdilution Method)

Antimicrobial Agent	MIC QC Ranges, µg/mL			
	Bacteroides fragilis ATCC®[a] 25285	*Bacteroides thetaiotaomicron* ATCC® 29741	*Clostridioides* (formerly *Clostridium*) *difficile* ATCC® 700057	*Eggerthella lenta* (formerly *Eubacterium lentum*) ATCC® 43055[b]
Amoxicillin-clavulanate (2:1)	0.25/0.125–1/0.5	0.25/0.125–1/0.5	–	–
Ampicillin-sulbactam (2:1)	0.5/0.25–2/1	0.5/0.25–2/1	–	0.5/0.25–2/1
Cadazolid	–	–	0.06–0.25	–
Cefotetan	1–8	16–128	–	16–64
Cefoxitin	2–8	8–64	–	2–16
Ceftaroline	2–16	8–64	0.5–4	–
Ceftaroline-avibactam	0.06/4–0.5/4	2/4–8/4	0.25/4–1/4	4/4–16/4
Ceftizoxime	–	–	–	8–32
Ceftolozane-tazobactam	0.12/4–1/4	16/4–64/4	–	–
Chloramphenicol	4–16	8–32	–	4–16
Clindamycin	0.5–2	2–8	–	0.06–0.25
Doripenem	0.12–0.5	0.12–1	–	–
Doxycycline	–	2–8	–	2–16
Eravacycline	0.016–0.12	0.06–0.25	0.016–0.06	–
Ertapenem	0.06–0.5	0.5–2	–	0.5–4
Faropenem	0.016–0.06	0.12–1	–	0.5–2
Garenoxacin	0.06–0.25	0.25–2	–	0.5–2
Imipenem	0.03–0.25	0.25–1	–	0.25–2
Imipenem-relebactam	0.03/4–0.125/4	–	–	–
Linezolid	2–8	2–8	–	0.5–2
Meropenem	0.03–0.25	0.06–0.5	–	0.125–1
Metronidazole	0.25–2	0.5–4	–	0.125–0.5
Moxifloxacin	0.12–0.5	1.0–8	–	0.12–0.5
Omadacycline[c]	0.12–1	0.25–1	0.06–0.25	0.06–5
Penicillin	8–32	8–32	–	–
Piperacillin	4–16	8–64	–	8–32

Table 5E. (Continued)

Antimicrobial Agent	MIC QC Ranges, µg/mL			
	Bacteroides fragilis ATCC®[a] 25285	*Bacteroides thetaiotaomicron* ATCC® 29741	*Clostridioides* (formerly *Clostridium*) *difficile* ATCC® 700057	*Eggerthella lenta* (formerly *Eubacterium lentum*) ATCC® 43055[b]
Piperacillin-tazobactam	0.03/4–0.25/4	2/4–16/4	–	8/4–32/4
Razupenem	0.03–0.25	0.12–0.5	0.06–0.5	0.12–0.5
Ridinilazole	–	–	0.12–0.5	–
Sulopenem	–	0.03–0.25	0.5–2	0.25–1
Surotomycin[d]	–	–	0.12–1	1–4
Ticarcillin-clavulanate	0.06/2–0.5/2	0.5/2–2/2	–	8/2–32/2
Tigecycline[c]	0.06–0.5	0.25–1	0.03–0.12	–

Abbreviations: ATCC®, American Type Culture Collection; MIC, minimal inhibitory concentration; QC, quality control.

Footnotes

a. ATCC® is a registered trademark of the American Type Culture Collection.

b. MIC variability with some agents has been reported with *E. lenta* ATCC® 43055; therefore, QC ranges have not been established for all antimicrobial agents with this organism.

c. For broth microdilution testing of omadacycline and tigecycline, when MIC panels are prepared, the medium must be prepared fresh on the day of use. The medium must be no greater than 12 hours old at the time the panels are made; however, the panels may then be frozen for later use.

d. QC ranges reflect MICs obtained when broth is supplemented with calcium to a final concentration of 50 µg/mL.

NOTE: For four-dilution ranges, results at the extremes of the acceptable range(s) should be suspect. Verify validity with data from other QC strains.

Table 5F. MIC Reference Guide to QC Frequency to Support Modifications to Antimicrobial Susceptibility Test Systems

This table summarizes the suggested QC frequency when modifications are made to antimicrobial susceptibility test systems (refer to CLSI EP23[1] and CLSI M52[2]). **Alternative approaches can be used as determined by IQCP. Refer to Appendix I for additional guidance on selection of QC strains and QC testing frequency.**

Test Modification	Recommended QC Frequency			Comments
	1 Day	5 Days	Daily or Per IQCP	
MIC test(s)				
Use new shipment or lot number.	X			
Expand dilution range.	X			**Example:** Convert from breakpoint to expanded range MIC panels.
Reduce dilution range.	X			**Example:** Convert from expanded dilution range to breakpoint panels.
Use new method (same company).			X	**Examples:** Convert from overnight to rapid MIC test. In addition, perform in-house verification studies.
Use new manufacturer of MIC test.			X	In addition, perform in-house verification studies.
Use new manufacturer of broth or agar.		X		
Addition of new antimicrobial agent to existing system			X	In addition, perform in-house verification studies.
Inoculum preparation				
Convert inoculum preparation/standardization to use of a device that has its own QC protocol.		X		**Example:** Convert from visual adjustment of turbidity to use of a photometric device for which a QC procedure is provided.
Convert inoculum preparation/standardization to a method that depends on user technique.			X	**Example:** Convert from visual adjustment of turbidity to another method that is not based on a photometric device.
Instrument/software				
Software update that affects AST results		X		Monitor all drugs, not just those implicated in software modification.
Repair of instrument that affects AST results	X			Depending on extent of repair (eg, critical component such as the photographic device), additional testing may be appropriate (eg, 5 d).

Abbreviations: AST, antimicrobial susceptibility testing; d, day(s); FDA, US Food and Drug Administration; **IQCP, individualized quality control plan;** MIC, minimal inhibitory concentration; QC, quality control.

Table 5F. (Continued)

NOTE 1: QC can be performed before or concurrent with testing patient isolates. Patient results can be reported for that day if QC results are within the acceptable limits.

NOTE 2: Manufacturers of commercial or in-house-prepared tests should follow their own internal procedures and applicable regulations.

NOTE 3: Acceptable MIC QC limits for FDA-cleared antimicrobial susceptibility tests may differ slightly from acceptable CLSI QC limits. Users of each device should use the manufacturer's procedures and QC limits as indicated in the instructions for use.

NOTE 4: For troubleshooting out-of-range results, refer to CLSI M07[3] and Table 5G. Additional information is available in Appendix C (eg, organism characteristics, QC testing recommendations).

NOTE 5: Broth, saline, and/or water used to prepare an inoculum does not need routine QC.

NOTE 6: Information in boldface type is new or modified since the previous edition.

References for Table 5F

[1] CLSI. *Laboratory Quality Control Based on Risk Management.* 2nd ed. CLSI guideline EP23™. Clinical and Laboratory Standards Institute; 2023.

[2] CLSI. *Verification of Commercial Microbial Identification and Antimicrobial Susceptibility Testing Systems.* 1st ed. CLSI guideline M52. Clinical and Laboratory Standards Institute; 2015.

[3] CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically.* 12th ed. CLSI standard M07. Clinical and Laboratory Standards Institute; 2024.

Table 5G. MIC Troubleshooting Guide

This table provides guidance for troubleshooting and corrective action for out-of-range QC, primarily using CAMHB for broth microdilution. Refer to CLSI M07[1] for additional information. Out-of-range QC tests are often the result of contamination or the use of an incorrect QC strain; corrective action should first include repeating the test with a pure culture of a freshly subcultured QC strain. If the issue is unresolved, this troubleshooting guide should be consulted regarding additional suggestions for troubleshooting out-of-range QC results and unusual clinical isolate results. In addition, see general corrective action outlined in CLSI M07[1] and notify manufacturers of potential product problems.

General Comment

(1) QC organism maintenance: Avoid repeated subcultures. Retrieve new QC strain from stock (refer to CLSI M07[1]). If using lyophilized strains, follow the maintenance recommendations of the manufacturer.

Antimicrobial Agent	QC Strain	Observation	Probable Cause	Comments/Suggested Actions
β-LACTAMS				
β-Lactam combination agents	*Acinetobacter baumannii* ATCC®[a] 13304 *Escherichia coli* ATCC® 35218 *E. coli* ATCC® 13353 *Klebsiella pneumoniae* ATCC® 700603 *K. pneumoniae* ATCC® BAA-1705™	MIC too low or susceptible for single β-lactam agent; in range for combination β-lactam agent	Spontaneous loss of the plasmid encoding the β-lactamase	Obtain new frozen or lyophilized stock culture. Use other routine QC strain (if available). These strains should be stored at −60°C or below, and frequent subcultures should be avoided. **NOTE:** *K. pneumoniae* ATCC® BAA-2814™ is stable and does not require QC integrity check.
β-Lactam combination agents	*A. baumannii* ATCC® 13304 *E. coli* ATCC® 35218 *E. coli* ATCC® 13353 *K. pneumoniae* ATCC® 700603 *K. pneumoniae* ATCC® BAA-1705™ *K. pneumoniae* ATCC® BAA-2814™	MIC too high or resistant for both the single β-lactam agent and the combination β-lactam agent	Antimicrobial agent is degrading.	Use alternative lot of test materials. Check storage and package integrity. Imipenem and clavulanate are especially labile.
Carbenicillin	*Pseudomonas aeruginosa* ATCC® 27853	MIC too high	QC strain develops resistance after repeated subculture.	See general comment (1) on QC organism maintenance. Prepare new subculture from the frozen or freeze-dried stock every 2 wk to prevent loss of viability.

Table 5G. (Continued)

Antimicrobial Agent	QC Strain	Observation	Probable Cause	Comments/Suggested Actions
β-LACTAMS (Continued)				
Cefotaxime-clavulanate Ceftazidime-clavulanate	*K. pneumoniae* ATCC® 700603	Negative ESBL test	Spontaneous loss of the plasmid encoding the β-lactamase	See general comment (1) on QC organism maintenance.
Carbapenems	*P. aeruginosa* ATCC® 27853	MIC too high	Zn^{2+} concentration in media is too high.	Use alternative lot.
Carbapenems	*P. aeruginosa* ATCC® 27853	MIC too high	Antimicrobial agent is degrading.	Use alternative lot. Check storage conditions and package integrity. Repeated imipenem QC results at the upper end of QC range with *P. aeruginosa* ATCC® 27853 may indicate deterioration of the drug.
Penicillin	*Staphylococcus aureus* ATCC® 29213	MIC too high	QC strain is a β-lactamase producer; overinoculation may yield increased MICs.	Repeat with a carefully adjusted inoculum.
Penicillins	Any	MIC too low	pH of media too low	Acceptable pH range = 7.2–7.4 Avoid CO_2 incubation, which lowers pH.
Penicillins	Any	MIC too high	pH of media too high	Acceptable pH range = 7.2–7.4
β-Lactam group	Any	MIC initially acceptable, but increases to possibly be out of range over time	Imipenem, cefaclor, and clavulanate are especially labile. Antimicrobial agents are degrading.	Use alternative lot. Check storage and package integrity.
NON-β-LACTAMS				
Aminoglycosides Quinolones	Any	MIC too high	pH of media too low	Acceptable pH range = 7.2–7.4 Avoid CO_2 incubation, which lowers pH.
Aminoglycosides Quinolones	Any	MIC too low	pH of media too high	Acceptable pH range = 7.2–7.4

Table 5G. (Continued)

Antimicrobial Agent	QC Strain	Observation	Probable Cause	Comments/Suggested Actions
NON-β-LACTAMS (Continued)				
Aminoglycosides	*P. aeruginosa* ATCC® 27853	MIC too low	Ca^{2+} and/or Mg^{2+} content too low	Acceptable range = Ca^{2+} 20–25 mg/L Mg^{2+} 10–12.5 mg/L
Aminoglycosides	*P. aeruginosa* ATCC® 27853	MIC too high	Ca^{2+} and/or Mg^{2+} content too high	Acceptable range = Ca^{2+} 20–25 mg/L Mg^{2+} 10–12.5 mg/L
Ceftriaxone	*P. aeruginosa* ATCC® 27853	MIC too high	QC strain develops resistance after repeated subculture.	See general comment (1) on QC organism maintenance. Prepare new subculture from the frozen or freeze-dried stock every 2 wk to prevent loss of viability.
Colistin[b]	*P. aeruginosa* ATCC® 27853 *E. coli* NCTC 13846 *E. coli* ATCC® BAA-3170™	MIC too high	Inadequate concentration of drug available in test medium due to drug adherence to surfaces (eg, tubes, plates)	Check composition of containers (eg, tubes, plates) used for production of test reagents and performance of MIC tests. Use tubes/plates made of untreated polystyrene. Prepare colistin stock solution on the day of use in production of tubes or panels for MIC testing. Use only the sulphate salts of polymyxins; the methanesulfonate derivative of colistin must not be used (it is an inactive prodrug that breaks down slowly in solution).
Colistin[b]	*P. aeruginosa* ATCC® 27853 *E. coli* NCTC 13846 *E. coli* ATCC® BAA-3170™	MIC too low	Surfactant added to test broth or inoculum diluent	Check to ensure surfactant (eg, polysorbate-80) was not added to test medium or inoculum diluent.
Dalbavancin Oritavancin (see CLSI M07[1]) Telavancin	*S. aureus* ATCC® 29213 *Enterococcus faecalis* ATCC® 29212	MIC too high	Lack of polysorbate-80 in the media	Add polysorbate-80 to CAMHB to final concentration of 0.002% (v/v). See CLSI M07[1] and Appendix A.

Table 5G. (Continued)

Antimicrobial Agent	QC Strain	Observation	Probable Cause	Comments/Suggested Actions
NON-β-LACTAMS (Continued)				
Chloramphenicol Clindamycin Erythromycin Linezolid Tedizolid Tetracycline	*S. aureus* ATCC® 29213 *E. faecalis* ATCC® 29212 *Streptococcus pneumoniae* ATCC® 49619	MIC too high	Trailing end point	Read at first well where the trailing begins; tiny buttons of growth should be ignored. See general comment (3) in Table 2G.
Linezolid Tedizolid	*S. aureus* ATCC® 29213	MIC too high	Trailing end point	*S. aureus* ATCC® 25923 may be used as a supplemental QC strain for these drugs. This strain exhibits less trailing and MIC end points are easier to interpret.
Oritavancin (see CLSI M07[1])	*S. aureus* ATCC® 29213 *E. faecalis* ATCC® 29212	MIC too high	Lack of polysorbate-80 in the solvent and diluent	Dissolve antimicrobial powder and prepare dilutions in water containing a final concentration of 0.002% polysorbate-80 (v/v).
Oritavancin	*S. aureus* ATCC® 29213 *E. faecalis* ATCC® 29212	MIC too high	Use of tissue-culture treated microdilution trays	Use only untreated microdilution trays for this antimicrobial agent.[2]
Clindamycin Macrolides Ketolides	*S. aureus* ATCC® 29213 *E. faecalis* ATCC® 29212	MIC too high	pH of media too low	Acceptable pH range = 7.2–7.4 Avoid CO_2 incubation, which lowers pH.
Clindamycin Macrolides Ketolides	*S. aureus* ATCC® 29213 *E. faecalis* ATCC® 29212	MIC too low	pH of media too high	Acceptable pH range = 7.2–7.4
Daptomycin	*S. aureus* ATCC® 29213 *E. faecalis* ATCC® 29212	MICs too high MICs too low	Ca^{2+} content too low Ca^{2+} content too high	Acceptable Ca^{2+} content 50 µg/mL in CAMHB
Tetracyclines	Any	MIC too low	pH of media too low	Acceptable pH range = 7.2–7.4
Tetracyclines	Any	MIC too high	pH of media too high	Acceptable pH range = 7.2–7.4 Avoid CO_2 incubation, which lowers pH.

Table 5G. (Continued)

Antimicrobial Agent	QC Strain	Observation	Probable Cause	Comments/Suggested Actions
NON-β-LACTAMS (Continued)				
Tetracyclines	Any	MIC too high	Ca^{2+} and/or Mg^{2+} content too high	Acceptable range = Ca^{2+} 20–25 mg/L Mg^{2+} 10–12.5 mg/L
Tetracyclines	Any	MIC too low	Ca^{2+} and/or Mg^{2+} content too low	Acceptable range = Ca^{2+} 20–25 mg/L Mg^{2+} 10–12.5 mg/L
Omadacycline Tigecycline	Any	MIC too high	CAMHB has not been freshly prepared.	Reference panels must be used or frozen within 12 h of CAMHB preparation.
ALL AGENTS				
Various	*S. pneumoniae* ATCC® 49619	MICs too low Light growth	Inoculum source plate too old and contains too many nonviable cells.	See general comment (1) on QC organism maintenance. Prepare new subculture from the frozen or freeze-dried stock every 2 wk to prevent loss of viability. Subculture QC strain and repeat QC test or retrieve new QC strain from stock. Plate used to prepare inoculum should be incubated 18-20 h.
Various	*E. coli* ATCC® 35218 *K. pneumoniae* ATCC® 700603	MIC too low	Spontaneous loss of the plasmid encoding the β-lactamase	See general comment (1) on QC organism maintenance.
Various	*E. faecalis* ATCC® 51299	MIC too low	QC strain loses resistance after repeated subculture.	See general comment (1) on QC organism maintenance. Prepare new subculture from the frozen or freeze-dried stock every 2 wk to prevent loss of viability.
Various	Any	One QC result is out of range, but the antimicrobial agent is not an agent reported for patient results (eg, not on hospital formulary).	N/A	If antimicrobial agent is not normally reported, no repeat is necessary if adequate controls are in place to prevent reporting of the out-of-range antimicrobial agent.

Table 5G. (Continued)

Antimicrobial Agent	QC Strain	Observation	Probable Cause	Comments/Suggested Actions
ALL AGENTS (Continued)				
Various	Any	Many MICs too low	Inoculum too light; error in inoculum preparation	Repeat using McFarland 0.5 turbidity standard or standardizing device. Check expiration date and proper storage if using barium sulfate or latex standards. Check steps in inoculum preparation and inoculation procedure. Perform colony count check of GC well immediately after inoculation and before incubation (*E. coli* ATCC® 25922 closely approximates 5 × 10^5 CFU/mL; see CLSI M07[1]).
Various	Any	Many MICs too high or too low	CAMHB not optimal	Use alternative lot.
Various	Any	Many MICs too high or too low	Possible reading/ transcription error	Recheck readings. Use alternative lot.
Various	Any	Many MICs too high	Inoculum too heavy	Repeat using McFarland 0.5 turbidity standard or standardizing device. Check expiration date and proper storage if using barium sulfate or latex standards. Check steps in inoculum preparation and inoculation procedure. Perform colony count check of GC well immediately after inoculation and before incubation (*E. coli* ATCC® 25922 closely approximates 5 × 10^5 CFU/mL; see CLSI M07[1]).

Table 5G. (Continued)

Antimicrobial Agent	QC Strain	Observation	Probable Cause	Comments/Suggested Actions
ALL AGENTS (Continued)				
Various	Any	Skipped wells	Contamination. Improper inoculation of panel or inadequate mixing of inoculum. Actual concentration of drug in wells inaccurate. Volume of broth in wells inaccurate.	Repeat QC test. Use alternative lot.
Various	Any	QC results from one strain are out of range, but other QC strains are in range with the same antimicrobial agent.	One QC organism may be a better indicator of a QC problem (eg, *P. aeruginosa* ATCC® 27853 is a better indicator of imipenem deterioration than *E. coli* ATCC® 25922).	Determine whether the in-range QC strain has an on-scale end point for the agent in question. Retest this strain to confirm reproducibility of acceptable results. Evaluate with alternative strains with known MICs. Initiate corrective action with problem QC strain/antimicrobial agent(s).
Various	Any	QC results from two strains are out of range with the same antimicrobial agent.	Indicates a problem with the antimicrobial agent. May be a systemic problem.	Initiate corrective action.
Various	Any	QC results from one strain are out of range, but the antimicrobial agent is not an agent reported for patient results (eg, not on hospital formulary).		If antimicrobial agent is not normally reported, no repeat is necessary if adequate controls are in place to prevent reporting of the out-of-range antimicrobial agent. Carefully check antimicrobial agents of the same class for similar trend toward out-of-control results. If the antimicrobial agent in question is consistently out of control, contact the manufacturer.

Abbreviations: ATCC®, American Type Culture Collection; CAMHB, cation-adjusted Mueller-Hinton broth; CFU, colony-forming unit(s); CO_2, carbon dioxide; ESBL, extended-spectrum β-lactamase; GC, growth control; h, hour(s); MIC, minimal inhibitory concentration; N/A, not applicable; NCTC, National Collection of Type Cultures; pH, negative logarithm of hydrogen ion concentration; QC, quality control; wk, week(s).

Footnotes

a. ATCC® is a trademark of the American Type Culture Collection.

b. Colistin results are significantly affected by preparation and handling of reagents and/or testing materials, including stock solutions, test medium, composition of testing tube and/or plate (eg, glass, polystyrene, polypropylene). QC results may fall outside the established CLSI QC ranges if methods other than CLSI reference methods described in CLSI M07[1] and CLSI M100 are used.

Table 5G. (Continued)

References for Table 5G

[1] CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically.* 12th ed. CLSI standard M07. Clinical and Laboratory Standards Institute; 2024.

[2] Arhin FF, Sarmiento I, Belley A, et al. Effect of polysorbate 80 on oritavancin binding to plastic surfaces: implications for susceptibility testing. *Antimicrob Agents Chemother.* 2008;52(5):1597-1603. doi:10.1128/AAC.01513-07

Table 6A. Solvents and Diluents for Preparing Stock Solutions of Antimicrobial Agents[a]

Antimicrobial Agent	Solvent[b] Unless otherwise stated, use a minimum amount of the listed solvent to solubilize the antimicrobial powder.	Diluent[b] Finish diluting the final stock solution as stated below.
Amikacin	Water	Water
Amoxicillin	Phosphate buffer, pH 6, 0.1 mol/L	Phosphate buffer, pH 6, 0.1 mol/L
Ampicillin	Phosphate buffer, pH 8, 0.1 mol/L	Phosphate buffer, pH 6, 0.1 mol/L
Avibactam	Water	Water
Azithromycin	95% ethanol or glacial acetic acid[a,c]	Broth media
Azlocillin	Water	Water
Aztreonam	Saturated solution sodium bicarbonate	Water
Besifloxacin	Methanol	Water
Biapenem	Saline[d]	Saline[d]
Cadazolid	DMSO[a]	Water or broth
Carbenicillin	Water	Water
Cefaclor	Water	Water
Cefadroxil	Phosphate buffer, pH 6, 0.1 mol/L	Water
Cefamandole	Water	Water
Cefazolin	Phosphate buffer, pH 6, 0.1 mol/L	Phosphate buffer, pH 6, 0.1 mol/L
Cefdinir	Phosphate buffer, pH 6, 0.1 mol/L	Water
Cefditoren	Phosphate buffer, pH 6, 0.1 mol/L	Water
Cefepime	Phosphate buffer, pH 6, 0.1 mol/L	Phosphate buffer, pH 6, 0.1 mol/L or water
Cefetamet	Phosphate buffer, pH 6, 0.1 mol/L	Water
Cefiderocol	Saline[d]	Saline[d]
Cefixime	Phosphate buffer, pH 7, 0.1 mol/L	Phosphate buffer, pH 7, 0.1 mol/L
Cefmetazole	Water	Water
Cefonicid	Water	Water
Cefoperazone	Water	Water
Cefotaxime	Water	Water
Cefotetan	DMSO[a]	Water

Table 6A. (Continued)

Antimicrobial Agent	Solvent[b]	Diluent[b]
	Unless otherwise stated, use a minimum amount of the listed solvent to solubilize the antimicrobial powder.	Finish diluting the final stock solution as stated below.
Cefoxitin	Water	Water
Cefpodoxime	0.10% (11.9 mmol/L) aqueous sodium bicarbonate	Water
Cefprozil	Water	Water
Ceftaroline	DMSO[a] to 30% of total volume	Saline[d]
Ceftazidime	Sodium carbonate[e]	Water
Ceftibuten	Phosphate buffer, pH 8, 0.1 mol/L	Water or phosphate buffer, pH 8, 0.1 mol/L
Ceftizoxime	Water	Water
Ceftobiprole	DMSO plus glacial acetic acid[a,f]	Water, vortex vigorously
Ceftolozane	Water or saline[d]	Water or saline[d]
Ceftriaxone	Water	Water
Cefuroxime	Phosphate buffer, pH 6, 0.1 mol/L	Phosphate buffer, pH 6, 0.1 mol/L
Cephalexin	Phosphate buffer, pH 6, 0.1 mol/L	Water
Cephalothin	Phosphate buffer, pH 6, 0.1 mol/L	Water
Cephapirin	Phosphate buffer, pH 6, 0.1 mol/L	Water
Cephradine	Phosphate buffer, pH 6, 0.1 mol/L	Water
Chloramphenicol	95% ethanol	Water
Cinoxacin	1/2 volume of water, then add 1 mol/L NaOH dropwise to dissolve	Water
Ciprofloxacin	Water	Water
Clarithromycin	Methanol[a] or glacial acetic acid[a,c]	Phosphate buffer, pH 6.5, 0.1 mol/L
Clavulanate	Phosphate buffer, pH 6, 0.1 mol/L	Phosphate buffer, pH 6, 0.1 mol/L
Clinafloxacin	Water	Water
Clindamycin	Water	Water
Colistin[g]	Water	Water
Dalbavancin	DMSO[a]	DMSO[a,h]
Daptomycin	Water	Water

Table 6A. (Continued)

Antimicrobial Agent	Solvent[b]	Diluent[b]
	Unless otherwise stated, use a minimum amount of the listed solvent to solubilize the antimicrobial powder.	Finish diluting the final stock solution as stated below.
Delafloxacin	1/2 volume of water, then 0.1 mol/L NaOH dropwise to dissolve	Water
Dirithromycin	Glacial acetic acid[c]	Water
Doripenem	Saline[d]	Saline[d]
Doxycycline	Water	Water
Durlobactam	Water	Water
Enoxacin	1/2 volume of water, then 0.1 mol/L NaOH dropwise to dissolve	Water
Enmetazobactam	Water	Water
Eravacycline	Water	Water
Ertapenem	Phosphate buffer, pH 7.2, 0.01 mol/L	Phosphate buffer, pH 7.2, 0.01 mol/L
Erythromycin	95% ethanol or glacial acetic acid[a,c]	Water
Exebacase	Supplied as a frozen stock in a buffer containing 20 mM L-histidine and 5% D-sorbitol, pH 7[i]	CAMHB-HSD[j]
Faropenem	Water	Water
Fidaxomicin	DMSO[a]	Water
Finafloxacin	Water	Water
Fleroxacin	1/2 volume of water, then 0.1 mol/L NaOH dropwise to dissolve	Water
Fosfomycin	Water	Water
Funobactam	DMSO[a]	Water or CAMHB
Fusidic acid	Water	Water
Garenoxacin	Water (with stirring)	Water
Gatifloxacin	Water (with stirring)	Water
Gemifloxacin	Water	Water
Gentamicin	Water	Water
Gepotidacin	DMSO[a]	Water
Iclaprim	DMSO[a]	Water

Table 6A. (Continued)

Antimicrobial Agent	Solvent[b] Unless otherwise stated, use a minimum amount of the listed solvent to solubilize the antimicrobial powder.	Diluent[b] Finish diluting the final stock solution as stated below.
Imipenem	Phosphate buffer, pH 7.2, 0.01 mol/L	Phosphate buffer, pH 7.2, 0.01 mol/L
Kanamycin	Water	Water
Ledaborbactam	Water	Water
Lefamulin	Water	Water
Levofloxacin	1/2 volume of water, then 0.1 mol/L NaOH dropwise to dissolve	Water
Levonadifloxacin	27.5 μg/mL solution of L-arginine in water	Water
Linezolid	Water	Water
Lomefloxacin	Water	Water
Loracarbef	Water	Water
Mecillinam	Water	Water
Meropenem	Water	Water
Metronidazole	DMSO[a]	Water
Minocycline	Water	Water
Moxalactam (diammonium salt)[k]	0.04 mol/L HCl (let sit for 1.5 to 2 h)	Phosphate buffer, pH 6, 0.1 mol/L
Moxifloxacin	Water	Water
Mupirocin	Water	Water
Nacubactam	Water	Water
Nafcillin	Water	Water
Nafithromycin	1/2 volume of water, then glacial acetic acid dropwise to dissolve (acetic acid not to exceed 2.5 μL/mL)	Water
Nalidixic acid	1/2 volume of water, then add 1 mol/L NaOH dropwise to dissolve	
Netilmicin	Water	Water
Nitazoxanide	DMSO[a,l]	DMSO[a,l]
Nitrofurantoin[m]	Phosphate buffer, pH 8, 0.1 mol/L	Phosphate buffer, pH 8, 0.1 mol/L
Norfloxacin	1/2 volume of water, then 0.1 mol/L NaOH dropwise to dissolve	Water

Table 6A. (Continued)

Antimicrobial Agent	Solvent[b] Unless otherwise stated, use a minimum amount of the listed solvent to solubilize the antimicrobial powder.	Diluent[b] Finish diluting the final stock solution as stated below.
Ofloxacin	1/2 volume of water, then 0.1 mol/L NaOH dropwise to dissolve	Water
Omadacycline	Water	Water
Oritavancin	0.002% polysorbate-80 in water[n]	0.002% polysorbate-80 in water[n]
Oxacillin	Water	Water
Ozenoxacin	10% volume of water, then 1M NaOH (8% of final volume)	Water
Penicillin	Water	Water
Pexiganan	Water	Water
Piperacillin	Water	Water
Plazomicin	Water	Water
Polymyxin B	Water	Water
Quinupristin-dalfopristin	Water	Water
Ramoplanin	Water	Water
Razupenem	Phosphate buffer, pH 7.2, 0.01 mol/L	Phosphate buffer, pH 7.2, 0.01 mol/L
Relebactam	Water	Water
Ridinilazole	DMSO[a]	DMSO[a]
Rifampin	Methanol[a] (maximum concentration = 640 µg/mL)	Water (with stirring)
Rifaximin	Methanol[a]	0.1 M phosphate buffer, pH 7.4 + 0.45% sodium dodecyl sulfate
Secnidazole	DMSO[a]	Water
Solithromycin	Glacial acetic acid[c]	Water
Sparfloxacin	Water	Water
Spectinomycin	Water	Water
Streptomycin	Water	Water
Sulbactam	Water	Water
Sulfonamides	1/2 volume hot water and minimal amount of 2.5 mol/L NaOH to dissolve	Water
Sulopenem[o]	0.01 M phosphate buffer, pH 7.2, vortex to dissolve	0.01 M phosphate buffer, pH 7.2

Table 6A. (Continued)

Antimicrobial Agent	Solvent[b]	Diluent[b]
	Unless otherwise stated, use a minimum amount of the listed solvent to solubilize the antimicrobial powder.	Finish diluting the final stock solution as stated below.
Surotomycin	Water	Water
Taniborbactam	Water	Water
Tazobactam	Water	Water
Tebipenem	Water	Water
Tedizolid	DMSO[a]	DMSO[a,p]
Teicoplanin	Water	Water
Telavancin	DMSO[a]	DMSO[a,h]
Telithromycin	Glacial acetic acid[a,c]	Water
Tetracycline	Water	Water
Ticarcillin	Phosphate buffer, pH 6, 0.1 mol/L	Phosphate buffer, pH 6, 0.1 mol/L
Ticarcillin-clavulanate	Phosphate buffer, pH 6, 0.1 mol/L	Phosphate buffer, pH 6, 0.1 mol/L
Tigecycline	Water	Water
Tinidazole	DMSO[a,l]	Water
Tizoxanide	DMSO[a,l]	DMSO[a,l]
Tobramycin	Water	Water
Trimethoprim	0.05 mol/L lactic acid[a] or HCl,[a] 10% of final volume	Water (may need heat)
Trimethoprim (if lactate)	Water	Water
Trospectomycin	Water	Water
Ulifloxacin (prulifloxacin)	DMSO[a]	Water
Upleganan	Water	Water
Vaborbactam	90% DMSO[a]/10% water	Water
Vancomycin	Water	Water
Xeruborbactam	Water	Water
Zidebactam	Water	Water
Zoliflodacin	DMSO	Water
Zosurabalpin	**Water**	**Water**

Abbreviations: CAMHB, cation-adjusted Mueller-Hinton broth; CAMHB-HSD, cation-adjusted Mueller-Hinton broth supplemented with horse serum (25% v/v) and 0.5 mM DL-dithiothreitol (pH 7.2–7.4); DMSO, dimethyl sulfoxide; h, hour(s); HCl, hydrochloric acid; NaCl, sodium chloride; NaOH, sodium hydroxide; pH, negative logarithm of hydrogen ion concentration.

Table 6A. (Continued)

Footnotes

a. Consult the safety data sheets before working with any antimicrobial reference standard powder, solvent, or diluent. Some of the compounds (eg, solvents such as DMSO, methanol) are more toxic than others and may necessitate handling in a chemical fume hood.

b. Although these solvents and diluents are recommended, users should always confirm with the manufacturer.

c. For glacial acetic acid, use 1/2 volume of water, then add glacial acetic acid dropwise until dissolved, not to exceed 2.5 µL/mL.

d. Saline – a solution of 0.85% to 0.9% NaCl (w/v).

e. Anhydrous sodium carbonate is used at a weight of exactly 10% of the ceftazidime to be used. The sodium carbonate is dissolved in solution in most of the necessary water. The antimicrobial agent is dissolved in this sodium carbonate solution, and water is added to the desired volume. The solution is to be used as soon as possible, but it can be stored up to 6 hours at no more than 25°C.

f. For each 1.5 mg of ceftobiprole, add 110 µL of a 10:1 mixture of DMSO and glacial acetic acid. Vortex vigorously for 1 minute, then intermittently for 15 minutes. Dilute to 1 mL with distilled water.

g. The formulation of colistin reference standard powder used in antimicrobial susceptibility tests is colistin sulfate and not colistin methane sulfonate (sulfomethate).

h. Starting stock solutions of dalbavancin and telavancin should be prepared at concentrations no higher than 1600 µg/mL. Intermediate 100× concentrations should then be diluted in DMSO. Final 1:100 dilutions should then be made directly into CAMHB supplemented with 0.002% (v/v) polysorbate-80, so the final concentration of DMSO in the wells is no greater than 1%. See also Table 8B.

i. Exebacase is an enzyme that requires special handling. **See Appendix H, section H2.**

j. **See Appendix H, section H2 for instructions for preparation** of CAMHB-HSD.

k. The diammonium salt of moxalactam is very stable, but it is almost pure R isomer. Moxalactam for clinical use is a 1:1 mixture of R and S isomers. Therefore, the salt is dissolved in 0.04 mol/L HCl and allowed to react for 1.5 to 2 hours to convert it to equal parts of both isomers.

l. Final concentration of DMSO should not exceed 1%. This may be accomplished as follows: 1) prepare the stock solution at 10 times higher concentration than planned stock solution (ie, prepare at 12 800 µg/mL, rather than 1280 µg/mL); 2) add 1.8 mL sterile water to each agar deep; 3) add 0.2 mL of each antibiotic dilution to each agar deep.

m. Alternatively, nitrofurantoin is dissolved in DMSO.

n. Starting stock solutions of oritavancin should be prepared at concentrations no higher than 1600 µg/mL in 0.002% polysorbate-80 in water. Intermediate 100× oritavancin concentrations should then be prepared in 0.002% polysorbate-80 in water. Final 1:100 dilutions should be made directly into CAMHB supplemented with 0.002% polysorbate-80, so the final concentration of polysorbate-80 in the wells is 0.002%.

Table 6A. (Continued)

o. Must be made fresh on the day of use.

p. Starting stock solutions of tedizolid should be prepared at concentrations no higher than 1600 µg/mL. Intermediate 100× concentrations should be diluted in DMSO. Final 1:100 dilutions should be made directly into CAMHB, so that the final concentration of DMSO in the wells is no greater than 1%. Also see Table 8B.

NOTE: Information in boldface type is new or modified since the previous edition.

Table 6B. Preparing Stock Solutions for Antimicrobial Agents Provided With Activity Expressed as Units

Antimicrobial Agent	Pure Agent	Calculation for µg/mg	Example
Potassium penicillin G	0.625 µg/unit[1]	Multiply the activity expressed in units/mg by 0.625 µg/unit.	Activity units/mg • 0.625 µg/unit = Activity µg/mg (eg, 1592 units/mg • 0.625 µg/unit = 995 µg/mg)
Sodium penicillin G	0.6 µg/unit[1]	Multiply the activity expressed in units/mg by 0.6 µg/unit.	Activity units/mg • 0.6 µg/unit = Activity µg/mg (eg, 1477 units/mg • 0.6 µg/unit = 886.2 µg/mg)
Polymyxin B	10 000 units/mg = 10 units/µg = 0.1 µg/unit[2]	Multiply the activity expressed in units/mg by 0.1 µg/unit.	Activity units/mg • 0.1 µg/unit = Activity µg/mg (eg, 8120 units/mg • 0.1 µg/unit = 812 µg/mg)
		Divide the activity expressed in units/mg by 10 units/µg.	Activity units/mg / 10 units/µg = Activity µg/mg (eg, 8120 units/mg / 10 units/mg = 812 µg/mg)
Colistin sulfate[a]	30 000 units/mg = 30 units/µg = 0.03333 µg/unit[2]	Multiply the activity expressed in units/mg by 0.03333 µg/unit.	Activity units/mg • 0.03333 µg/unit = Activity µg/mg (eg, 20 277 units/mg • 0.03333 µg/unit = 676 µg/mg)
		Divide the activity expressed in units/mg by 30 units/µg.	Activity units/mg / 30 units/µg = Activity µg/mg (eg, 20 277 units/mg / 30 units/µg = 676 µg/mg)
Streptomycin	785 units/mg[3]	Divide the number of units given for the powder by 785. This gives the percent purity of the powder. Multiply the percent purity by 850, which is the amount in the purest form of streptomycin. This result equals the activity factor in µg/mg.	([Potency units/mg] / [785 units/mg]) • (850 µg/mg) = Potency µg/mg (eg, [751 units/mg / 785 units/mg] • 850 µg/mg = 813 µg/mg) If powder contains 2.8% water: 813 • (1 – 0.028) = potency 813 • 0.972 = 790 µg/mg

Footnote

a. Do not use colistin methanesulfonate for *in vitro* antimicrobial susceptibility tests.

Table 6B. (Continued)

References for Table 6B

1 Geddes AM, Gould IM. Benzylpenicillin (penicillin G). In: Grayson ML, ed. *Kucers' The Use of Antibiotics: A Clinical Review of Antibacterial, Antifungal, Antiparasitic and Antiviral Drugs.* 6th ed. CRC Press, Taylor & Francis Group; 2010:5-58.

2 Polymyxins. In: Kucers A, Crowe SM, Grayson ML, Hoy JF, eds. *The Use of Antibiotics: A Clinical Review of Antibacterial, Antifungal and Antiviral Drugs.* 5th ed. Butterworth-Heinemann; 1997:667-675.

3 US Department of Agriculture, Food Safety and Inspection Service, Office of Public Health Science, Laboratory QA/QC Division. *Bioassay for the Detection, Identification and Quantitation of Antimicrobial Residues in Meat and Poultry Tissue.* MLG 34.03. US Department of Agriculture; 2011.

Table 6C. Preparing Solutions and Media Containing Combinations of Antimicrobial Agents

Antimicrobial Agent	Combination Tested	Preparation	Example
Amikacin-fosfomycin	5:2 ratio (amikacin:fosfomycin)	Prepare 10× starting concentration as 5:2 ratio and dilute as needed. **NOTE:** Media should be supplemented with 25 µg/mL glucose-6-phosphate.	
Amoxicillin-clavulanate	2:1 ratio (amoxicillin:clavulanate)	Prepare 10× starting concentration as 2:1 ratio and dilute as needed.	For a starting concentration of 128/64 in the panel, prepare a 10× stock concentration of 2560 µg/mL for amoxicillin and 1280 µg/mL for clavulanate. Then combine equal amounts of each to the first dilution tube, which will then contain 1280/640 µg/mL of the combination. Dilute 1:10 with broth to achieve the final concentration in microdilution wells.
Ampicillin-sulbactam	2:1 ratio (ampicillin:sulbactam)	Same as amoxicillin-clavulanate.	
Aztreonam-avibactam	Fixed concentration of avibactam at 4 µg/mL	Prepare 10× starting concentration of aztreonam at twice the concentration needed and dilute as usual using serial 2-fold dilutions. Add an equal volume of avibactam 80 µg/mL to each of the diluted tubes.	For a starting concentration of 128/4 in the panel, prepare a 10× stock concentration of aztreonam at 2560 µg/mL and dilute by serial 2-fold increments down to the final concentration needed in the panel. Prepare a stock concentration of avibactam at 80 µg/mL. Then add an equal volume of the avibactam 80 µg/mL solution to each diluted tube of aztreonam. For example, 5 mL of 2560 µg/mL aztreonam + 5 mL of 80 µg/mL avibactam = 10 mL of 1280/40 µg/mL aztreonam-avibactam. Dilute 1:10 with broth to achieve the final concentration in microdilution wells.
Aztreonam-nacubactam	1:1 ratio (aztreonam:nacubactam)	Prepare 10× starting concentration as 1:1 ratio and dilute as needed.	For a starting concentration of 128/128 in the panel, prepare a 20× stock concentration of 2560 µg/mL for aztreonam and 2560 µg/mL for nacubactam. Combine equal amounts of each to the first dilution tube, which will then contain 1280/1280 µg/mL of the combination. Prepare 2-fold serial dilutions and dilute each 1:10 with broth to achieve the final concentration in the microdilution wells.

Table 6C. (Continued)

Antimicrobial Agent	Combination Tested	Preparation	Example
Cefepime-enmetazobactam	Fixed concentration of enmetazobactam at 8 mg/L	Prepare 10× starting concentration of cefepime at twice the concentration needed and dilute as usual using serial 2-fold dilutions. Add an equal volume of enmetazobactam 160 µg/mL to each of the diluted tubes.	For a starting concentration of 128/8 in the panel, prepare a 10× stock concentration of cefepime at 2560 µg/mL and dilute by serial 2-fold increments down to the final concentration needed in the panel. Prepare a stock concentration of enmetazobactam at 160 µg/mL. Then add an equal volume of the enmetazobactam 160 µg/mL solution to each diluted tube of cefepime. For example, 5 mL of 2560 µg/mL cefepime + 5 mL of 160 µg/mL enmetazobactam = 10 mL of 1280/80 µg/mL cefepime-enmetazobactam. Dilute 1:10 with broth to achieve the final concentration in the microdilution wells.
Cefepime-nacubactam	1:1 ratio (cefepime:nacubactam)	Prepare 10× starting concentration as 1:1 ratio and dilute as needed.	For a starting concentration of 128/128 in the panel, prepare a 20× stock concentration of 2560 µg/mL for cefepime and 2560 µg/mL for nacubactam. Combine equal amounts of each to the first dilution tube, which will then contain 1280/1280 µg/mL of the combination. Prepare 2-fold serial dilutions and dilute each 1:10 with broth to achieve the final concentration in the microdilution wells.
Cefepime-taniborbactam	Fixed concentration of taniborbactam at 4 µg/mL	Prepare 10× starting concentration of cefepime at twice the concentration needed and dilute as usual using serial 2-fold dilutions. Add an equal volume of taniborbactam 80 µg/mL to each of the diluted tubes.	For a starting concentration of 128/4 in the panel, prepare a 10× stock concentration of cefepime at 2560 µg/mL and dilute by serial 2-fold increments down to the final concentration needed in the panel. Prepare a stock concentration of taniborbactam at 80 µg/mL. Then add an equal volume of the taniborbactam 80 µg/mL solution to each diluted tube of cefepime. For example, 5 mL of 2560 µg/mL cefepime + 5 mL of 80 µg/mL taniborbactam = 10 mL of 1280/40 µg/mL cefepime-taniborbactam. Dilute 1:10 with broth to achieve the final concentration in microdilution wells.

Table 6C. (Continued)

Antimicrobial Agent	Combination Tested	Preparation	Example
Cefepime-tazobactam	Fixed concentration of tazobactam at 8 µg/mL	Prepare 10× starting concentration of cefepime at twice the concentration needed and dilute as usual using serial 2-fold dilutions. Add an equal volume of tazobactam 160 µg/mL to each of the diluted tubes.	For a starting concentration of 128/8 in the panel, prepare a 10× stock concentration of cefepime at 2560 µg/mL and dilute by serial 2-fold increments down to the final concentration needed in the panel. Prepare a stock concentration of tazobactam at 160 µg/mL. Then add an equal volume of the tazobactam 160 µg/mL solution to each diluted tube of cefepime. For example, 5 mL of 2560 µg/mL cefepime + 5 mL of 160 µg/mL tazobactam = 10 mL of 1280/80 µg/mL cefepime-tazobactam. Dilute 1:10 with broth to achieve the final concentration in the microdilution wells.
Cefepime-zidebactam	1:1 ratio (cefepime:zidebactam)	Prepare 10× starting concentration as 1:1 ratio and dilute as needed.	For a starting concentration of 128/128 in the panel, prepare a 20× stock concentration of 2560 µg/mL for cefepime and 2560 µg/mL for zidebactam. Then combine equal amounts of each to the first dilution tube, which will then contain 1280/1280 µg/mL of the combination. Prepare 2-fold serial dilutions and dilute each 1:10 with broth to achieve the final concentration in the microdilution wells.
Ceftaroline-avibactam	Fixed concentration of avibactam at 4 µg/mL	Same as aztreonam-avibactam.	
Ceftazidime-avibactam	Fixed concentration of avibactam at 4 µg/mL	Same as aztreonam-avibactam.	
Ceftibuten-avibactam	Fixed concentration of avibactam at 4 µg/mL	Same as aztreonam-avibactam.	
Ceftibuten-ledaborbactam	Fixed concentration of ledaborbactam at 4 µg/mL	Same as aztreonam-avibactam.	
Ceftibuten-xeruborbactam	**Fixed concentration of xeruborbactam at 4 µg/mL**	**Prepare 10× starting concentration of ceftibuten at twice the concentration needed and dilute as usual using serial 2-fold dilutions. Add an equal volume of xeruborbactam 80 µg/mL to each of the diluted tubes.**	**For a starting concentration of 64/4 µg/mL in the MIC panels, prepare a 10× stock concentration of ceftibuten at 1280 µg/mL and dilute by serial 2-fold increments down to the final concentration needed in the panel. Prepare a stock concentration of xeruborbactam at 80 µg/mL. Then add an equal volume of the xeruborbactam 80 µg/mL solution to each diluted tube of ceftibuten. For example, 5 mL of 1280 µg/mL ceftibuten + 5 mL of 80 µg/mL xeruborbactam = 10 mL of 640/40 µg/mL ceftibuten-xeruborbactam. Dilute 1:10 with CAMHB to achieve the final concentration in the MIC panel wells.**

Table 6C. (Continued)

Antimicrobial Agent	Combination Tested	Preparation	Example
Ceftolozane-tazobactam	Fixed concentration of tazobactam at 4 µg/mL	Same as aztreonam-avibactam.	
Imipenem-funobactam	Fixed concentration of funobactam at 8 µg/mL	Prepare 10× starting concentration of imipenem at twice the concentration needed and dilute as usual using serial 2-fold dilutions. Add an equal volume of funobactam 160 µg/mL to each of the diluted tubes.	For a starting concentration of 16/8 µg/mL in the panel, prepare a 10× stock concentration of imipenem at 320 µg/mL and dilute by serial 2-fold increments down to the final concentration needed in the panel. Prepare a stock concentration of funobactam at 160 µg/mL. Then add an equal volume of the funobactam 160 µg/mL solution to each diluted tube of imipenem. For example, 5 mL of 320 µg/mL imipenem + 5 mL of 160 µg/mL funobactam = 10 mL of 160/80 µg/mL imipenem-funobactam. Dilute 1:10 with broth to achieve the final concentration in the microdilution wells.
Imipenem-relebactam	Fixed concentration of relebactam at 4 µg/mL	Same as aztreonam-avibactam.	
Meropenem-nacubactam	1:1 ratio (meropenem:nacubactam)	Prepare 10× starting concentration as 1:1 ratio and dilute as needed.	For a starting concentration of 128/128 in the panel, prepare a 20× stock concentration of 2560 µg/mL for meropenem and 2560 µg/mL for nacubactam. Combine equal amounts of each to the first dilution tube, which will then contain 1280/1280 µg/mL of the combination. Prepare 2-fold serial dilutions and dilute each 1:10 with broth to achieve the final concentration in the microdilution wells.
Meropenem-vaborbactam	Fixed concentration of vaborbactam at 8 µg/mL	Prepare 10× starting concentration of meropenem at twice the concentration needed and dilute as usual using serial 2-fold dilutions. Add an equal volume of vaborbactam 160 µg/mL to each of the diluted tubes.	For a starting concentration of 64/8 µg/mL in the panel, prepare a 10× stock concentration of meropenem at 1280 µg/mL and dilute by serial 2-fold increments down to the final concentration needed in the panel. Prepare a stock concentration of vaborbactam at 160 µg/mL. Then add an equal volume of the vaborbactam 160 µg/mL solution to each diluted tube of meropenem. For example, 5 mL of 1280 µg/mL meropenem + 5 mL of 160 µg/mL vaborbactam = 10 mL of 640/80 µg/mL meropenem-vaborbactam. Dilute 1:10 with broth to achieve the final concentration in the microdilution wells.

Table 6C. (Continued)

Antimicrobial Agent	Combination Tested	Preparation	Example
Meropenem-xeruborbactam	Fixed concentration of xeruborbactam at 8 µg/mL	Prepare 10× starting concentration of meropenem at twice the concentration needed and dilute as usual using serial 2-fold dilutions. Add an equal volume of xeruborbactam 160 µg/mL to each of the diluted tubes.	For a starting concentration of 64/8 µg/mL in the panel, prepare a 10× stock concentration of meropenem at 1280 µg/mL and dilute by serial 2-fold increments down to the final concentration needed in the panel. Prepare a stock concentration of xeruborbactam at 160 µg/mL. Then add an equal volume of the xeruborbactam 160 µg/mL solution to each diluted tube of meropenem. For example, 5 mL of 1280 µg/mL meropenem + 5 mL of 160 µg/mL xeruborbactam = 10 mL of 640/80 µg/mL meropenem-xeruborbactam. Dilute 1:10 with broth to achieve the final concentration in the microdilution wells.
Piperacillin-tazobactam	Fixed concentration of tazobactam at 4 µg/mL	Same as aztreonam-avibactam.	
Sulbactam-durlobactam	Fixed concentration of durlobactam at 4 µg/mL	Prepare 10× starting concentration of sulbactam at twice the concentration needed and dilute as usual using serial 2-fold dilutions. Add an equal volume of durlobactam 80 µg/mL to each of the diluted tubes.	For a starting concentration of 128/4 in the panel, prepare a 10× stock concentration of sulbactam at 2560 µg/mL and dilute by serial 2-fold increments down to the final concentration needed. Prepare a stock concentration of durlobactam at 80 µg/mL. Then add an equal volume of the durlobactam 80 µg/mL solution to each diluted tube of sulbactam. For example, 5 mL of 2560 µg/mL sulbactam + 5 mL of 80 µg/mL durlobactam = 10 mL of 1280/40 µg/mL sulbactam-durlobactam. Dilute 1:10 with broth to achieve the final concentration in microdilution wells.
Ticarcillin-clavulanate	Fixed concentration of clavulanate at 2 µg/mL	Prepare 10× starting concentration of ticarcillin at twice the concentration needed and dilute as usual using serial 2-fold dilutions. Add an equal volume of clavulanate 40 µg/mL to each of the diluted tubes.	For a starting concentration of 128/2 in the panel, prepare a 10× stock concentration of ticarcillin at 2560 µg/mL and dilute by serial 2-fold increments down to the final concentration needed. Prepare a stock concentration of clavulanate at 40 µg/mL. Then add an equal volume of the clavulanate 40 µg/mL solution to each diluted tube of ticarcillin. For example, 5 mL of 2560 µg/mL ticarcillin + 5 mL of 40 µg/mL clavulanate = 10 mL of 1280/20 µg/mL ticarcillin-clavulanate. Dilute 1:10 with broth to achieve the final concentration in microdilution wells.

Table 6C. (Continued)

Antimicrobial Agent	Combination Tested	Preparation	Example
Trimethoprim-sulfamethoxazole	1:19 ratio (trimethoprim:sulfamethoxazole)	Prepare a 10× starting concentration of trimethoprim at 1600 µg/mL (or at 1280 µg/mL that will need dilution to 160 µg/mL). Prepare a 10× starting concentration of sulfamethoxazole at a $\log_2$ multiple of 1520 µg/mL (eg, 1520, 3040, or 6080 µg/mL) depending on the starting concentration needed.	For a starting concentration of 8/152 in the panel, prepare a 10× concentration of trimethoprim at 160 µg/mL. Prepare a 10× starting concentration of sulfamethoxazole at 3040 µg/mL. Add an equal volume of the 160 µg/mL trimethoprim and the 3040 µg/mL sulfamethoxazole to the first dilution tube, and then dilute by serial 2-fold dilutions as usual. For example, 5 mL of 160 µg/mL trimethoprim and 5 mL of 3040 µg/mL sulfamethoxazole = 10 mL of 80/1520 trimethoprim-sulfamethoxazole. Dilute 1:10 with broth to achieve the final concentration in microdilution wells.
Quinupristin-dalfopristin	Preparation usually not necessary, because drug powder is received as combination.		

Abbreviations: CAMBH, cation-adjusted Mueller-Hinton broth; MIC, minimal inhibitory concentration.

NOTE 1: To prepare intermediate dilutions of antimicrobial agents, a convenient formula to use is $C_1 \cdot V_1 = C_2 \cdot V_2$, where C_1 is the concentration of stock solution of the antimicrobial agent (usually 1280 µg/mL or greater); V_1 is the unknown volume that will be needed to make the intermediate concentration; C_2 is the intermediate concentration needed; and V_2 is the volume of the intermediate stock solution needed. For example, to prepare 20 mL of a 40 µg/mL solution from a 1280 µg/mL stock solution:

$$C_1 \cdot V_1 = C_2 \cdot V_2$$

$$1280\ \mu g/mL \cdot V_1 = 40\ \mu g/mL \cdot 20\ mL$$

$$V_1 = 40\ \mu g/mL \cdot 20\ mL\ /\ 1280\ \mu g/mL$$

$$V_1 = 0.625\ mL$$

Therefore, add 0.625 mL of the 1280 µg/mL stock solution to 19.375 mL of diluent (usually water) for a final volume of 20 mL of a 40 µg/mL solution.

NOTE 2: Information in boldface type is new or modified since the previous edition.

Table 7. Preparing Dilutions of Antimicrobial Agents to Be Used in Agar Dilution Susceptibility Tests

Antimicrobial Solution										
Step	Concentration, µg/mL	Source	Volume, mL	+	Diluent, mL	=	Intermediate Concentration, µg/mL	=	Final Concentration at 1:10 Dilution in Agar, µg/mL	Log_2
	5120	Stock	–		–		5120		512	9
1	5120	Stock	2		2		2560		256	8
2	5120	Stock	1		3		1280		128	7
3	5120	Stock	1		7		640		64	6
4	640	Step 3	2		2		320		32	5
5	640	Step 3	1		3		160		16	4
6	640	Step 3	1		7		80		8	3
7	80	Step 6	2		2		40		4	2
8	80	Step 6	1		3		20		2	1
9	80	Step 6	1		7		10		1	0
10	10	Step 9	2		2		5		0.5	–1
11	10	Step 9	1		3		2.5		0.25	–2
12	10	Step 9	1		7		1.25		0.125	–3

Abbreviation: MIC, minimal inhibitory concentration.

NOTE: This table is modified from Ericsson HM, Sherris JC. Antibiotic sensitivity testing: report of an international collaborative study. *Acta Pathol Microbiol Scand B Microbiol Immunol*. 1971;217(suppl 217):1+.

When serial twofold dilution MICs are being prepared and tested, the actual dilution scheme is:

128, 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25, 0.125, 0.0625, 0.03125, 0.015625, 0.0078125, 0.0039063, 0.0019531 µg/mL, etc.

For convenience only, and not because these are the actual concentrations tested, it was decided to use the following values in these tables:

128, 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25, 0.12, 0.06, 0.03, 0.016, 0.008, 0.004, 0.002 µg/mL, etc.

The values that appear in the tables are equivalent to the actual values tested, eg, 0.12 µg/mL = 0.125 µg/mL, 0.016 µg/mL = 0.015625 µg/mL.

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Table 8A. Preparing Dilutions of Antimicrobial Agents to Be Used in Broth Dilution Susceptibility Tests

Antimicrobial Solution								
Step	Concentration,[a] µg/mL	Source	Volume[a], mL	+	CAMHB[b] Volume,[c] mL	=	Final Concentration, µg/mL	Log_2
1	5120	Stock	1		9		512	9
2	512	Step 1	1		1		256	8
3	512	Step 1	1		3		128	7
4	512	Step 1	1		7		64	6
5	64	Step 4	1		1		32	5
6	64	Step 4	1		3		16	4
7	64	Step 4	1		7		8	3
8	8	Step 7	1		1		4	2
9	8	Step 7	1		3		2	1
10	8	Step 7	1		7		1	0
11	1	Step 10	1		1		0.5	-1
12	1	Step 10	1		3		0.25	-2
13	1	Step 10	1		7		0.125	-3

Abbreviations: CAMHB, cation-adjusted Mueller-Hinton broth; MIC, minimal inhibitory concentration.

Footnotes

a. See Table 7 for the dilution scheme when serial twofold dilution MICs are being prepared and tested.

b. Adjustment with cations, if necessary, occurs before this step.

c. The volumes selected can be any multiple of these figures, depending on the number of tests to be performed.

NOTE: This table is modified from Ericsson HM, Sherris JC. Antibiotic sensitivity testing: report of an international collaborative study. *Acta Pathol Microbiol Scand B Microbiol Immunol.* 1971;217(suppl 217):1+.

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Table 8B. Preparing Dilutions of Water-Insoluble Antimicrobial Agents to Be Used in Broth Dilution Susceptibility Tests

Antimicrobial Solution										
Step	Concentration, µg/mL	Source	Volume, mL	+	Solvent, mL (eg, DMSO)	=	Intermediate Concentration, µg/mL	=	Final Concentration at 1:100, µg/mL	Log$_2$
1	1600	Stock					1600		16	4
2	1600	Stock	0.5		0.5		800		8.0	3
3	1600	Stock	0.5		1.5		400		4.0	2
4	1600	Stock	0.5		3.5		200		2.0	1
5	200	Step 4	0.5		0.5		100		1.0	0
6	200	Step 4	0.5		1.5		50		0.5	–1
7	200	Step 4	0.5		3.5		25		0.25	–2
8	25	Step 7	0.5		0.5		12.5		0.125	–3
9	25	Step 7	0.5		1.5		6.25		0.0625	–4
10	25	Step 7	0.5		3.5		3.1		0.03	–5
11	3.1	Step 10	0.5		0.5		1.6		0.016	–6
12	3.1	Step 10	0.5		1.5		0.8		0.008	–7
13	3.1	Step 10	0.5		3.5		0.4		0.004	–8
14	0.4	Step 13	0.5		0.5		0.2		0.002	–9

Abbreviation: DMSO, dimethyl sulfoxide.

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Appendix A. Suggestions for Confirming Antimicrobial Susceptibility Test Results and Organism Identification for Agents Approved by the US Food and Drug Administration for Clinical Use

Organism or Organism Group	Antimicrobial Class/Subclass	Antimicrobial Agents and Resistance Phenotypes Detected[a]	Occurrence and Significance of Resistance and Actions to Take Following Confirmation of Results[a]		
			Category I	**Category II**	**Category III**
			Not reported or only rarely reported to date	**Uncommon in most institutions**	**May be common but generally considered of epidemiological concern**
			Action Steps:		
			• Confirm ID and susceptibility.[a] • Report to infection prevention. • Check with public health department to determine appropriate reporting and isolate referral procedures. • Save isolate. **NOTE:** It may be appropriate to notify infection prevention of preliminary findings before confirmation of results.	• Confirm ID and susceptibility if uncommon in the institution.[a] • Check with infection prevention in the facility to determine whether special reporting procedures or additional actions are needed. • Check with public health department to determine appropriate reporting and isolate referral procedures.	• Confirm ID and susceptibility if uncommon in the institution.[a] • Check with infection prevention in the facility to determine whether special reporting procedures or additional action are needed.
Any Enterobacterales	β-Lactam combination agents	Ceftazidime-avibactam – R Imipenem-relebactam – I or R Meropenem-vaborbactam – I or R		X	
	Cephems	Cefiderocol – I or R	X		
	Carbapenems	Any carbapenem – I or R[b]		X	
	Aminoglycosides	Amikacin, gentamicin, and tobramycin – R			X
		Plazomicin – R (except *Proteus mirabilis*)	X		
	Lipopeptides	Colistin/polymyxin B – R[c]	X		

Appendix A. (Continued)

Organism or Organism Group	Antimicrobial Class/Subclass	Antimicrobial Agents and Resistance Phenotypes Detected[a]	Occurrence and Significance of Resistance and Actions to Take Following Confirmation of Results[a]		
			Category I	Category II	Category III
			Not reported or only rarely reported to date	Uncommon in most institutions	May be common but generally considered of epidemiological concern
Escherichia coli, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, and *P. mirabilis*	Cephems	Cephalosporin III/IV – I/SDD or R			X
Salmonella and *Shigella* spp.[d]	Cephems	Cephalosporin III – I or R		X	
	Macrolides	Azithromycin – R		X	
	Fluoroquinolones	Any fluoroquinolone – I or R		X	
Pseudomonas aeruginosa	β-Lactam combination agents	Ceftazidime-avibactam – R Ceftolozane-tazobactam – I or R Imipenem-relebactam – I or R			X
	Cephems	Cefiderocol – I or R	X		
	Carbapenems	Any carbapenem[c] – I or R			X
	Aminoglycosides	Amikacin and tobramycin – R			X
	Lipopeptides	Colistin/polymyxin B – R	X		
Acinetobacter baumannii complex	**β-Lactam combination agents**	**Sulbactam-durlobactam – I or R**	**X**		
	Cephems	Cefiderocol – I or R	X		
	Carbapenems	Any carbapenem[c] – I or R			X
	Lipopeptides	Colistin/polymyxin B – R	X		
Stenotrophomonas maltophilia	Cephems	Cefiderocol – NS	X		
	Folate pathway antagonists	Trimethoprim-sulfamethoxazole – I or R			X

Appendix A. (Continued)

Organism or Organism Group	Antimicrobial Class/Subclass	Antimicrobial Agents and Resistance Phenotypes Detected[a]	Occurrence and Significance of Resistance and Actions to Take Following Confirmation of Results[a]		
			Category I	Category II	Category III
			Not reported or only rarely reported to date	Uncommon in most institutions	May be common but generally considered of epidemiological concern
Staphylococcus aureus	Penicillinase-stable penicillins	Oxacillin – R			X
	Cephems	Ceftaroline – SDD or R		X	
	Glycopeptides	Vancomycin – I[e]		X	
		Vancomycin – R	X		
	Lipoglycopeptides	Dalbavancin – NS Oritavancin – NS Telavancin – NS	X		
	Lipopeptides	Daptomycin – NS		X	
	Streptogramins	Quinupristin-dalfopristin (MSSA only) – I or R		X	
	Oxazolidinones	Linezolid – R Tedizolid – I or R		X	
	Pleuromutilins	Lefamulin – NS	X		
Staphylococcus spp. other than *Staphylococcus aureus* (SOSA)	Glycopeptides	Vancomycin – I or R[f]		X	
	Lipopeptides	Daptomycin – NS		X	
	Oxazolidinones	Linezolid – R		X	

Appendix A. (Continued)

Organism or Organism Group	Antimicrobial Class/Subclass	Antimicrobial Agents and Resistance Phenotypes Detected[a]	Occurrence and Significance of Resistance and Actions to Take Following Confirmation of Results[a]		
			Category I	Category II	Category III
			Not reported or only rarely reported to date	Uncommon in most institutions	May be common but generally considered of epidemiological concern
Enterococcus spp.	Glycopeptides	Vancomycin – R[c]			X
	Lipoglycopeptides (Vancomycin-susceptible *E. faecalis* only)	Dalbavancin – NS Oritavancin – NS Telavancin – NS	X		
	Lipopeptides	Daptomycin – I or R		X	
	Oxazolidinones	Linezolid – R Tedizolid – NS		X	
	Aminoglycosides	Gentamicin high level – R Streptomycin high level – R			X
Haemophilus influenzae	Penicillins	Ampicillin – R and β-lactamase negative		X	
	β-Lactam combination agents	Amoxicillin-clavulanate – R		X	
		Ceftolozane-tazobactam – NS	X		
	Cephems	Cephalosporin III/IV – NS Ceftaroline – NS	X		
	Carbapenems	Any carbapenem – NS	X		
	Fluoroquinolones	Any fluoroquinolone – NS	X		
	Pleuromutilins	Lefamulin – NS	X		
Neisseria gonorrhoeae	Cephems	Cephalosporin III/IV – NS		X	
	Macrolides	Azithromycin – NS			X
	Fluoroquinolones	Ciprofloxacin – I or R			X

Appendix A. (Continued)

Organism or Organism Group	Antimicrobial Class/Subclass	Antimicrobial Agents and Resistance Phenotypes Detected[a]	Occurrence and Significance of Resistance and Actions to Take Following Confirmation of Results[a]		
			Category I	**Category II**	**Category III**
			Not reported or only rarely reported to date	**Uncommon in most institutions**	**May be common but generally considered of epidemiological concern**
Streptococcus pneumoniae	Penicillins	Amoxicillin or penicillin (nonmeningitis) – R			X
	Cephems	Cephalosporin III/IV (nonmeningitis) – R			X
		Ceftaroline (nonmeningitis) – NS	X		
	Carbapenems	Any carbapenem – I, R, or NS		X	
	Glycopeptides	Vancomycin – NS	X		
	Fluoroquinolones	Any fluoroquinolone – I or R		X	
	Streptogramins	Quinupristin-dalfopristin – I or R		X	
	Ansamycins	Rifampin – I or R		X	
	Oxazolidinones	Linezolid – NS	X		
	Pleuromutilins	Lefamulin – NS	X		
Streptococcus, β-hemolytic group	Penicillins	Ampicillin or penicillin – NS	X		
	Cephems	Cephalosporin III/IV – NS	X		
		Ceftaroline – NS			
	Carbapenems	Any carbapenem – NS	X		
	Glycopeptides	Vancomycin – NS	X		
	Lipoglycopeptides	Dalbavancin – NS	X		
		Oritavancin – NS	X		
		Telavancin – NS	X		
	Lipopeptides	Daptomycin – NS	X		
	Streptogramins	Quinupristin-dalfopristin (*S. pyogenes* only) – I or R		X	
	Oxazolidinones	Linezolid – NS	X		
		Tedizolid – NS	X		

Appendix A. (Continued)

Organism or Organism Group	Antimicrobial Class/Subclass	Antimicrobial Agents and Resistance Phenotypes Detected[a]	Occurrence and Significance of Resistance and Actions to Take Following Confirmation of Results[a]		
			Category I	Category II	Category III
			Not reported or only rarely reported to date	Uncommon in most institutions	May be common but generally considered of epidemiological concern
Streptococcus, viridans group	Carbapenems	Any carbapenem – NS	X		
	Glycopeptides	Vancomycin – NS	X		
	Lipoglycopeptides	Dalbavancin (*S. anginosus* group only) – NS	X		
		Oritavancin – NS	X		
		Telavancin – NS	X		
	Streptogramins	Quinupristin-dalfopristin – I or R	X		
	Oxazolidinones	Linezolid – NS	X		
		Tedizolid – NS	X		
Neisseria meningitidis	Penicillins	Ampicillin or penicillin – I		X	
		Ampicillin or penicillin – R	X		
	Cephems	Cephalosporin III – NS	X		
	Carbapenems	Meropenem – NS	X		
	Macrolides	Azithromycin – NS		X	
	Tetracyclines	Minocycline – NS		X	
	Fluoroquinolones	Any fluoroquinolone – I or R		X	
	Phenicols	Chloramphenicol – I or R		X	
	Ansamycins	Rifampin – I or R		X	
Bacteroides and *Parabacteroides* spp.	β-Lactam combination agents	Imipenem-relebactam – I or R		X	
	Carbapenems	Any carbapenem – I or R		X	
	Nitroimidazoles	Metronidazole – I or R		X	

Abbreviations: Carba NP, carbapenemase Nordmann-Poirel; FDA, US Food and Drug Administration; I, intermediate; ID, identification; mCIM, modified carbapenem inactivation method; MIC, minimal inhibitory concentration; MSSA, methicillin (oxacillin)-susceptible *Staphylococcus aureus*; NS, nonsusceptible; R, resistant; SDD, susceptible-dose dependent; **SOSA, staphylococci other than *Staphylococcus aureus*.**

Appendix A. (Continued)

Footnotes

a. Ensure antimicrobial susceptibility test results and organism identification are accurate and reproducible. Consider the following steps:
   1. Check for transcription errors, contamination, or defective panel, plate, or card.
   2. Check previous reports on the patient to determine if the isolate was encountered and confirmed earlier.
   3. Repeat organism identification and antimicrobial susceptibility tests with initial method to ensure they reproduce. For category I and II, the laboratory may elect to skip step 3 and go to steps 4 and 5. For category III, repeat and/or confirmatory testing may not be needed if resistance is common in the institution.
   4. Confirm organism identification with second method performed in-house or at a referral laboratory.
   5. Confirm antimicrobial susceptibility test results with second method (eg, in-house or referral laboratory). The second method might be a CLSI reference method (eg, broth microdilution, agar dilution, or disk diffusion) or an FDA-cleared commercial test.

b. Imipenem MICs for *Proteus* spp., *Providencia* spp., and *Morganella morganii* tend to be higher (eg, MICs in the intermediate or resistant category) than meropenem or doripenem MICs. MICs for imipenem may be elevated due to mechanisms other than carbapenemases among these organisms. A phenotypic test such as Carba NP or mCIM may be used to identify carbapenemase-producing isolates (see Tables 3B and 3C).

c. Excludes organisms with intrinsic resistance to listed agents as described in Appendix B.

d. When submitting the report to a public health department, include antimicrobial susceptibility test results for *Salmonella* spp. that are intermediate or resistant to third-generation cephalosporins (cephalosporin III) and/or intermediate or resistant to fluoroquinolone or resistant to nalidixic acid.

e. *S. aureus* isolates demonstrating vancomycin MICs 4 µg/mL may represent testing variation and need not be reported or submitted to public health department; *S. aureus* isolates demonstrating MICs > 4 µg/mL should be reported to the local public health department.

f. There are some *Staphylococcus* spp. other than *S. aureus* for which vancomycin MICs may test within the intermediate range (MIC 8–16 µg/mL). In contrast, vancomycin-resistant *Staphylococcus* spp. (MIC ≥ 32 µg/mL) are rare.

NOTE 1: NS: A category used for isolates for which only a susceptible interpretive criterion has been designated because of the absence or rare occurrence of resistant strains. Isolates that have MICs above or zone diameters below the value indicated for the susceptible breakpoint should be reported as nonsusceptible.

NOTE 2: An isolate that is interpreted as nonsusceptible does not necessarily mean that the isolate has a resistance mechanism. It is possible that isolates with MICs above the susceptible breakpoint that lack resistance mechanisms may be encountered within the wild-type distribution subsequent to the time the susceptible-only breakpoint is set.

Appendix A. (Continued)

NOTE 3: For strains yielding results in the "nonsusceptible" category, organism identification and antimicrobial susceptibility test results should be confirmed (see footnote a).

NOTE 4: Information in boldface type is new or modified since the previous edition.

Appendix B. Intrinsic Resistance

Intrinsic resistance is defined as inherent or innate (not acquired) antimicrobial resistance, which is reflected in wild-type antimicrobial patterns of all or almost all representatives of a species. Intrinsic resistance is so common that susceptibility testing is unnecessary. For example, *Citrobacter* spp. are intrinsically resistant to ampicillin.

These tables can be helpful in at least three ways: 1) they provide a way to evaluate the accuracy of testing methods; 2) they aid in the recognition of common phenotypes; and 3) they can assist with verification of cumulative antimicrobial susceptibility test data. In the tables, an “R” occurring with an antimicrobial agent–organism combination means that strains should test resistant. A small percentage (1% to 3%) may appear susceptible due to method variation, mutation, or low levels of resistance expression.

Each laboratory should decide which agents to test and report in consultation with the antimicrobial stewardship team and other relevant institutional stakeholders. If tested, the result for an antimicrobial agent–organism combination listed as having intrinsic resistance should be reported as resistant. Consideration may be given to adding comments regarding intrinsic resistance of agents not tested. See Appendix A, footnote a.

B1. Enterobacterales

Antimicrobial Agent → **Organism ↓**	**Ampicillin**	**Amoxicillin-clavulanate**	**Ampicillin-sulbactam**	**Ticarcillin**	**Cephalosporins I: Cefazolin, Cephalothin**	**Cephamycins: Cefoxitin, Cefotetan**	**Cephalosporins II: Cefuroxime**	**Imipenem**	**Tetracyclines**	**Tigecycline**	**Nitrofurantoin**	**Polymyxin B Colistin**	**Aminoglycosides**
Citrobacter freundii	R	R	R		R	R	R						
Citrobacter koseri, Citrobacter amalonaticus group[a]	R			R									
Enterobacter cloacae complex[b]	R	R	R		R	R							
Escherichia coli	There is no intrinsic resistance to β-lactams in this organism.												
Escherichia hermannii	R			R									
Hafnia alvei	R	R	R		R	R						R[c]	
Klebsiella (formerly *Enterobacter*) *aerogenes*	R	R	R		R	R							
Klebsiella pneumoniae, Klebsiella oxytoca, Klebsiella variicola	R			R									
Morganella morganii	R	R			R		R	[d]		R	R	R	
Proteus mirabilis	There is no intrinsic resistance to penicillins and cephalosporins in this organism.							[d]	R	R	R	R	
Proteus penneri	R				R		R	[d]	R	R	R	R	

Appendix B. (Continued)

B1. Enterobacterales (Continued)

Antimicrobial Agent → Organism ↓	Ampicillin	Amoxicillin-clavulanate	Ampicillin-sulbactam	Ticarcillin	Cephalosporins I: Cefazolin, Cephalothin	Cephamycins: Cefoxitin, Cefotetan	Cephalosporins II: Cefuroxime	Imipenem	Tetracyclines	Tigecycline	Nitrofurantoin	Polymyxin B Colistin	Aminoglycosides
Proteus vulgaris	R				R		R	[d]	R	R	R	R	
Providencia rettgeri	R	R			R			[d]	R	R	R	R	
Providencia stuartii	R	R			R			[d]	R	R	R	R	[e]
Raoultella spp.[f]	R			R									
Salmonella and *Shigella* spp.	There is no intrinsic resistance to β-lactams in these organisms; refer to **WARNING** below for reporting.												
Serratia marcescens	R	R	R		R	R	R				R	R	
Yersinia enterocolitica	R	R		R	R								

Abbreviations: AST, antimicrobial susceptibility testing; MIC, minimal inhibitory concentration; R, resistant.

WARNING: For *Salmonella* and *Shigella* spp., aminoglycosides, first- and second-generation cephalosporins, and cephamycins may appear active *in vitro* but are not effective clinically and should not be reported as susceptible.

Footnotes

a. *C. amalonaticus* group includes *C. amalonaticus, Citrobacter farmeri,* and *Citrobacter sedlakii*.

b. *E. cloacae* complex includes *Enterobacter asburiae, E. cloacae,* and *Enterobacter hormaechei*. Other members of the complex include *Enterobacter kobei* and *Enterobacter ludwigii*, for which AST data are not available.

c. Colistin and polymyxin B resistance also applies to *Hafnia paralvei*.

d. *Proteus*, *Providencia*, and *Morganella* spp. may have elevated MICs to imipenem by mechanisms other than by production of carbapenemases. Isolates that test as susceptible should be reported as susceptible.

e. *P. stuartii* should be considered resistant to gentamicin, netilmicin, and tobramycin but not intrinsically resistant to amikacin.

f. *Raoultella* spp. include *Raoultella ornithinolytica, Raoultella terrigena,* and *Raoultella planticola*.

Appendix B. (Continued)

NOTE 1: Cephalosporins III, cefepime, cefiderocol, aztreonam, ticarcillin-clavulanate, piperacillin-tazobactam, imipenem-relebactam, ceftazidime-avibactam, meropenem-vaborbactam, and carbapenems are not listed because there is no intrinsic resistance in Enterobacterales.

NOTE 2: Enterobacterales are also intrinsically resistant to clindamycin, daptomycin, fusidic acid, glycopeptides (vancomycin), lipoglycopeptides (oritavancin, teicoplanin, telavancin), linezolid, tedizolid, quinupristin-dalfopristin, rifampin, and macrolides (erythromycin, clarithromycin, and azithromycin). However, there are some exceptions with macrolides (eg, *Salmonella* and *Shigella* spp. with azithromycin).

B2. Non-Enterobacterales

Antimicrobial Agent → **Organism ↓**	**Ampicillin, amoxicillin**	**Piperacillin**	**Ticarcillin**	**Ampicillin-sulbactam**	**Amoxicillin-clavulanate**	**Piperacillin-tazobactam**	**Cefotaxime**	**Ceftriaxone**	**Ceftazidime**	**Cefepime**	**Aztreonam**	**Imipenem**	**Meropenem**	**Ertapenem**	**Polymyxin B** **Colistin**	**Aminoglycosides**	**Tetracyclines** **Tigecycline**	**Trimethoprim**	**Trimethoprim-sulfamethoxazole**	**Chloramphenicol**	**Fosfomycin**
Acinetobacter baumannii/ Acinetobacter calcoaceticus complex	R				R						R			R				R		R	R
Burkholderia cepacia complex[a]	R	R	R	R	R	[a]	[a]	[a]		[a]	[a]	[a]		R	R	[a]		[a]			R
Pseudomonas aeruginosa	R			R	R		R	R						R			R	R	R	R	
Stenotrophomonas maltophilia	R	R	R	R	R	R	R	R			R	R	R	R		R	[b]	R			R

Abbreviations: MIC, minimal inhibitory concentration; R, resistant.

Footnotes

a. *B. cepacia* complex isolates have chromosomal genes that must undergo mutational changes before expressing resistance. It is not known how often these mutations occur during growth. Intrinsic resistance implies the presence of resistance mechanisms in natural or wild-type strains that result in phenotypic resistance for all or nearly all strains. Environmental *B. cepacia* complex strains lacking mutations do not express resistance mechanisms, resulting in low MICs to many antimicrobial agents, whereas clinical strains that express resistance genes, such as those from cystic fibrosis patients, have high MIC values to these same antimicrobial agents. There is insufficient clinical evidence to confirm whether strains that test susceptible *in vitro*, despite the presence of resistance mechanisms, will respond *in vivo*. Therefore, intrinsic resistance to the footnoted antibiotics (listed as resistant in previous editions of CLSI M100) cannot be confirmed.

b. *S. maltophilia* is intrinsically resistant to tetracycline but not to doxycycline, minocycline, or tigecycline.

Appendix B. (Continued)

NOTE: These nonfermentative gram-negative bacteria are also intrinsically resistant to penicillin (ie, benzylpenicillin), cephalosporins I (cephalothin, cefazolin), cephalosporin II (cefuroxime), cephamycins (cefoxitin, cefotetan), clindamycin, daptomycin, fusidic acid, glycopeptides (vancomycin), linezolid, macrolides (erythromycin, azithromycin, clarithromycin), quinupristin-dalfopristin, and rifampin.

B3. *Staphylococcus* spp.

Antimicrobial Agent → Organism ↓	Novobiocin	Fosfomycin	Fusidic acid
S. aureus *S. lugdunensis*	There is no intrinsic resistance in these species.		
S. epidermidis			
S. haemolyticus			
S. saprophyticus	R	R	R
S. capitis		R	
S. cohnii	R		
S. xylosus	R		

Abbreviations: MRS, methicillin (oxacillin)-resistant staphylococci; R, resistant.

NOTE 1: These gram-positive bacteria are also intrinsically resistant to aztreonam, polymyxin B/colistin, and nalidixic acid.

NOTE 2: MRS, as defined by cefoxitin or oxacillin testing, as appropriate to the species, are considered resistant to other β-lactam agents, ie, penicillins, β-lactam combination agents, cephems with the exception of ceftaroline, and carbapenems. This is because most cases of documented MRS infections have responded poorly to β-lactam therapy, or because convincing clinical data that document clinical efficacy for those agents have not been presented.

Appendix B. (Continued)

B4. *Enterococcus* spp.

Antimicrobial Agent → Organism ↓	Cephalosporins	Vancomycin	Teicoplanin	Aminoglycosides	Clindamycin	Quinupristin-dalfopristin	Trimethoprim	Trimethoprim-sulfamethoxazole	Fusidic acid
E. faecalis	R[a]			R[a]	R[a]	R	R	R[a]	R
E. faecium	R[a]			R[a]	R[a]		R	R[a]	R
E. gallinarum/E. casseliflavus	R[a]	R		R[a]	R[a]	R	R	R[a]	R

Abbreviation: R, resistant.

Footnote

a. **WARNING:** For *Enterococcus* spp., cephalosporins, aminoglycosides (except for high-level resistance testing), clindamycin, and trimethoprim-sulfamethoxazole may appear active *in vitro* but are not effective clinically and should not be reported as susceptible.

NOTE: These gram-positive bacteria are also intrinsically resistant to aztreonam, polymyxin B/colistin, and nalidixic acid.

Appendix B. (Continued)

B5. Anaerobic Gram-Positive Bacilli

Antimicrobial Agent → Organism ↓	Vancomycin	Aminoglycosides
Clostridium and *Clostridioides* spp.		R
Clostridium innocuum	R	R

Abbreviation: R, resistant.

B6. Anaerobic Gram-Negative Bacilli

Antimicrobial Agent → Organism ↓	Aminoglycosides	Penicillin	Ampicillin	Quinolones
Bacteroides spp.	R	R	R	
Fusobacterium canifelinum	R			R

Abbreviation: R, resistant.

Appendix C. QC Strains for Antimicrobial Susceptibility Tests

QC Strain	Organism Characteristics	Disk Diffusion Tests	MIC Tests	Other Tests	Comments
Acinetobacter baumannii NCTC 13304[a,b]	OXA-27 (carbapenemase)	β-Lactam combination agents	β-Lactam combination agents		
Bacteroides fragilis ATCC®[c] 25285	β-Lactamase positive		All anaerobes		
Bacteroides thetaiotaomicron ATCC® 29741	β-Lactamase positive		All anaerobes		
Clostridioides (formerly *Clostridium*) *difficile* ATCC® 700057	β-Lactamase negative		Gram-positive anaerobes		
Eggerthella lenta (formerly *Eubacterium lentum*) ATCC® 43055			All anaerobes		• Growth on Brucella medium not optimal • No longer required when establishing new QC ranges due to organism variability
Enterococcus faecalis ATCC® 29212			Nonfastidious gram-positive bacteria	• Vancomycin agar • HLAR tests • High-level mupirocin resistance MIC test	• Assess suitability of medium for sulfonamide or trimethoprim MIC and disk diffusion tests.[d] • Assess suitability of cation content in each batch/lot of MHB for daptomycin broth microdilution. Agar dilution has not been validated for daptomycin.
E. faecalis ATCC® 33186					Alternative to *E. faecalis* ATCC® 29212 to assess suitability of MHA for sulfonamide or trimethoprim disk diffusion tests[d]

Appendix C. (Continued)

QC Strain	Organism Characteristics	Disk Diffusion Tests	MIC Tests	Other Tests	Comments
E. faecalis ATCC® 51299	• *vanB* (vancomycin resistant) • Resistant to high-level aminoglycosides			• Vancomycin agar • HLAR tests	
Escherichia coli ATCC® 25922	β-Lactamase negative	• Nonfastidious gram-negative bacteria • *Neisseria meningitidis*	• Nonfastidious gram-negative bacteria • *N. meningitidis*		
E. coli ATCC® 35218[a,b,1]	TEM-1	β-Lactam combination agents	β-Lactam combination agents		
E. coli NCTC 13353[a,b,2]	• CTX-M-15 (ESBL) • OXA-1	β-Lactam combination agents	β-Lactam combination agents		
E. coli NCTC 13846	MCR-1		Nonfastidious gram-negative bacteria		
E. coli ATCC® BAA-3170™ (formerly *E. coli* AR Bank #0349 *mcr-1*)[3]	MCR-1		Nonfastidious gram-negative bacteria	• Colistin broth disk elution • Colistin agar test	
E. coli AR Bank #0348[3]			Nonfastidious gram-negative bacteria	Aztreonam plus ceftazidime-avibactam broth disk elution	Resistant to aztreonam, ceftazidime-avibactam, and aztreonam plus ceftazidime-avibactam in combination
Haemophilus influenzae ATCC® 10211					Assess each batch/lot of HTM for growth capabilities.
H. influenzae ATCC® 49247	BLNAR	• *H. influenzae* • *Haemophilus parainfluenzae*	• *H. influenzae* • *H. parainfluenzae*		
H. influenzae ATCC® 49766	Ampicillin susceptible	• *H. influenzae* • *H. parainfluenzae*	• *H. influenzae* • *H. parainfluenzae*		More reproducible than *H. influenzae* ATCC® 49247 with selected β-lactam agents

Appendix C. (Continued)

QC Strain	Organism Characteristics	Disk Diffusion Tests	MIC Tests	Other Tests	Comments
Klebsiella pneumoniae ATCC® 700603[a,b,1,4]	• SHV-18 (ESBL) • OXA-2 • Mutations in OMPK35 and OMPK37	β-Lactam combination agents	β-Lactam combination agents	ESBL tests	May demonstrate 2 colony morphologies: 1) opaque and cream colored and 2) translucent. Both colony morphologies can be used.
K. pneumoniae ATCC® BAA-1705™[a,b]	• KPC-2 (carbapenemase) • TEM • SHV	β-Lactam combination agents	β-Lactam combination agent	Carbapenemase tests	
K. pneumoniae ATCC® BAA-1706™	Resistant to carbapenems by noncarbapenemase mechanism			Carbapenemase tests	
K. pneumoniae ATCC® BAA-2146™	NDM			Carbapenemase tests	
K. pneumoniae ATCC® BAA-2814™[a,b] (previously B21 [KP1074])	• KPC-3 (carbapenemase) • SHV-11 • TEM-1	β-Lactam combination agents	β-Lactam combination agents		Higher MIC (see Table 5A-2) and better indicator of antimicrobial agent stability than *K. pneumoniae* BAA-1705™
Neisseria gonorrhoeae ATCC® 49226	CMRNG	*N. gonorrhoeae*	*N. gonorrhoeae*		
Pseudomonas aeruginosa ATCC® 27853[e]	Inducible AmpC β-lactamase	Nonfastidious gram-negative bacteria	Nonfastidious gram-negative bacteria		Assess suitability of cation content in each batch/lot of CAMHB.
Staphylococcus aureus ATCC® 25923	• β-Lactamase negative • *mecA* negative • *mupA* negative	Nonfastidious gram-positive bacteria		• High-level mupirocin resistance disk diffusion test • ICR disk diffusion test (D-zone test)	Little value in MIC testing due to its extreme susceptibility to most drugs

Appendix C. (Continued)

QC Strain	Organism Characteristics	Disk Diffusion Tests	MIC Tests	Other Tests	Comments
S. aureus ATCC® 29213	• Weak β-lactamase–producing strain • *mecA* negative • *mupA* negative		Nonfastidious gram-positive bacteria	• Oxacillin salt agar • High-level mupirocin resistance MIC test • ICR MIC test • Penicillin zone-edge test	Assess suitability of cation content in each batch/lot of MHB for daptomycin broth microdilution.
S. aureus ATCC® 43300	*mecA* positive	Cefoxitin disk diffusion testing	• Cefoxitin MIC testing • Oxacillin MIC testing	Oxacillin salt agar	
S. aureus ATCC® BAA-976™	*msr*[A]-mediated macrolide-only resistance			ICR MIC test and disk approximation test (D-zone test)	
S. aureus ATCC® BAA-977™	Inducible *erm*[A]-mediated macrolide resistance			ICR MIC test and disk approximation test (D-zone test)	
S. aureus ATCC® BAA-1708™	*mupA*-mediated high-level mupirocin resistance			High-level mupirocin resistance test	
Streptococcus pneumoniae ATCC® 49619	Penicillin intermediate by altered penicillin-binding protein	• *S. pneumoniae* • *Streptococcus* spp. • *N. meningitidis*	• *S. pneumoniae* • *Streptococcus* spp. • *N. meningitidis*	ICR MIC test	

Abbreviations: AR, antimicrobial resistance; ATCC®, American Type Culture Collection; BLNAR, β-lactamase negative, ampicillin-resistant; CAMHB, cation-adjusted Mueller-Hinton broth; CMRNG, chromosomally mediated penicillin-resistant *Neisseria gonorrhoeae*; ESBL, extended-spectrum β-lactamase; HLAR, high-level aminoglycoside resistance; HTM, *Haemophilus* test medium; ICR, inducible clindamycin resistance; **IQCP, individualized quality control plan;** MHA, Mueller-Hinton agar; MHB, Mueller-Hinton broth; MIC, minimal inhibitory concentration; NCTC, National Collection of Type Cultures; QC, quality control.

Appendix C. (Continued)

Footnotes

a. Careful attention to organism maintenance (eg, minimal subcultures) and storage (eg, −60°C or below) is especially important for these QC strains because spontaneous loss of the plasmid encoding the β-lactamase has been documented. If stored at temperatures above −60°C or if repeatedly subcultured, these strains may lose their resistance characteristics and QC results may be outside the acceptable ranges.

b. To confirm the integrity of the QC strain, test one of the single β-lactam agents highlighted in orange in Tables 4A-2 and 5A-2 by either a disk diffusion or MIC test when the strain is first subcultured from a frozen or lyophilized stock culture. In-range results for the single agent indicate the QC strain is reliable for QC of β-lactam combination agents. It is not necessary to check the QC strain again with a single agent until a new frozen or lyophilized stock culture is put into use.

c. ATCC® is a registered trademark of the American Type Culture Collection. Per ATCC® convention, the trademark symbol is used after "BAA" in each catalog number, in conjunction with the registered ATCC® name.

d. Disk diffusion and MIC end points should be easy to read as 80% or greater reduction in growth if the medium has acceptable levels of thymidine.

e. May develop resistance to β-lactam antimicrobial agents after repeated subcultures. Minimize this risk by subculturing from a frozen or lyophilized stock culture at least monthly or whenever the strain demonstrates results outside the acceptable range.

NOTE 1: QC strains **for routine QC** are tested regularly (ie, daily or **per IQCP**) to ensure the test system is working and produces results that fall within specified ranges listed in CLSI M100. The QC strains recommended in this document should be included if a laboratory performs CLSI disk diffusion or reference MIC testing as described herein. For commercial test systems, manufacturer's recommendations should be followed for all QC procedures. Some QC strains, **referred to as supplemental in CLSI M100**, are used to assess particular characteristics of a test or may represent alternative QC strains. For example, *H. influenzae* ATCC® 10211 is more fastidious than *H. influenzae* ATCC® 49247 or *H. influenzae* ATCC® 49766 and is used to ensure HTM can adequately support the growth of patient isolates of *H. influenzae* and *H. parainfluenzae*. **QC strains referred to as supplemental might be recommended for routine QC of some tests and supplemental QC for other tests**. QC strains may possess susceptibility or resistance characteristics specific for one or more special tests listed in CLSI M02[5] and CLSI M07.[6] **QC strains for supplemental QC** can be used to assess a new test, for training new personnel, and for competence assessment.

NOTE 2: Information in boldface type is new or modified since the previous edition.

Appendix C. (Continued)

References for Appendix C

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2. Woodford N, Ward ME, Kaufmann ME, et al. Community and hospital spread of *Escherichia coli* producing CTX-M extended-spectrum β-lactamases in the UK. *J Antimicrob Chemother.* 2004;54(4):735-743. doi:10.1093/jac/dkh424

3. Centers for Disease Control and Prevention. CDC & FDA Antibiotic Resistance Isolate Bank. Accessed 15 October 2024. https://wwwn.cdc.gov/ARIsolateBank/

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5. CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests.* 14th ed. CLSI standard M02. Clinical and Laboratory Standards Institute; 2024.

6. CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically.* 12th ed. CLSI standard M07. Clinical and Laboratory Standards Institute; 2024.

Appendix D. Anaerobe Cumulative Antibiogram

NOTE: Isolates collected from selected US hospitals from 1 January 2013 to 31 December 2016.[a]

D1. *Bacteroides* spp. and *Parabacteroides* spp.

Anaerobic Organisms	Number of Strains	Ampicillin-sulbactam		Number of Strains	Piperacillin-tazobactam		Number of Strains	Cefoxitin		Number of Strains	Ertapenem		Number of Strains	Imipenem		Number of Strains	Meropenem	
Percent susceptible (%S) and percent resistant (%R)[b]		%S	%R		%S	%R		%S	%R		%S	%R		%S	%R		%S	%R
Breakpoints, µg/mL		≤ 8/4	≥ 32/16		≤ 16/4	≥ 128/4		≤ 16	≥ 64		≤ 4	≥ 16		≤ 4	≥ 16		≤ 4	≥ 16
B. fragilis	129	84	2	1030	96	1	830	100	0	133	82	14	189	97	1	1505	93	5
B. thetaiotaomicron	76	82	5	502	87	0	508	13	54	–	–	–	70	100	0	328	99	0
B. ovatus	30	80	3	206	94	0	177	20	34	19[c]	84[c]	16[c]	49	100	0	236	95	1
B. vulgatus	20[c]	45[c]	15[c]	168	92	0	153	73	14	–	–	–	35	97	0	171	96	4
B. uniformis	19[c]	84[c]	0[c]	78	96	0	72	85	10	–	–	–	19[c]	100[c]	0[c]	93	100	0
P. distasonis	27[c]	59[c]	19[c]	92	95	1	82	29	43	–	–	–	26[c]	100[c]	0	119	97	2

Anaerobic Organisms	Number of Strains	Clindamycin		Number of Strains	Moxifloxacin		Number of Strains	Metronidazole	
Percent susceptible (%S) and percent resistant (%R)[b]		%S	%R		%S	%R		%S	%R
Breakpoints, µg/mL		≤ 2	≥ 8		≤ 2	≥ 8		≤ 8	≥ 32
B. fragilis	1013	26	22	256	61	32	1140	100	0
B. thetaiotaomicron	328	28	49	70	54	36	322	100	0
B. ovatus	207	46	51	59	41	25	236	100	0
B. vulgatus	171	53	46	29[c]	31[c]	45[c]	186	100	0
B. uniformis	87	45	48	25[c]	48[c]	40[c]	89	100	0
P. distasonis	108	43	44	37	62	35	118	100	0

Appendix D. (Continued)

Footnotes

a. Data were generated from unique isolates from patient specimens submitted to Tufts Medical Center, Boston, Massachusetts; International Health Management Associates, Inc., Schaumburg, Illinois; R.M. Alden Research Laboratory, Culver City, California; Creighton University School of Medicine, Omaha, Nebraska; Mayo Clinic College of Medicine and Science, Rochester, Minnesota; and the Centers for Disease Control and Prevention, Atlanta, Georgia. All testing was performed by the agar dilution method. Information and analysis of previous versions of this table have been published.

b. Intermediate category is not shown but can be derived by subtraction of %S and %R for each antimicrobial agent from %100.

c. Calculated from fewer than the CLSI M39[1] recommendation of 30 isolates.

D2. Anaerobic Organisms Other Than *Bacteroides* spp. and *Parabacteroides* spp.

Anaerobic Organisms	Number of Strains	Ampicillin-sulbactam		Number of Strains	Piperacillin-tazobactam		Number of Strains	Imipenem		Number of Strains	Meropenem		Number of Strains	Penicillin	
Percent susceptible (%S) and percent resistant (%R)[b]		%S	%R		%S	%R		%S	%R		%S	%R		%S	%R
Breakpoints, µg/mL		≤ 8/4	≥ 32/16		≤ 32/4	≥ 128/4		≤ 4	≥ 16		≤ 4	≥ 16		≤ 0.5	≥ 2
Prevotella spp.	29[c]	97[c]	3[c]	63	100	0	29[c]	100	0	92	98	0	63	100	0
Fusobacterium spp.	20[c]	100[c]	0[c]	55	96	2	75	95	4	20[c]	100[c]	0[c]	–[d]	–[d]	–[d]
Anaerobic gram-positive cocci[e]	–[d]	–[d]	–[d]	1853	99	1	134	99	0	1647	100	0	1647	100	0
Cutibacterium (formerly *Propionibacterium*) *acnes*[f]	–[d]	–[d]	–[d]	18[c]	100[c]	0[c]	17[c]	94[c]	0[d]	–[d]	–[d]	–[d]	–[d]	–[d]	–[d]
Clostridium perfringens	15[c]	100[c]	0	410	100	0	23[c]	100[c]	0[c]	417	100	0	402	90	4
Clostridioides (formerly *Clostridium*) *difficile*[g]	76	99	0	542	93	0	480	69	4	609	99	0	533	6	37
Other *Clostridium* spp.	–[d]	–[d]	–[d]	439	94	1	71	99	0	390	100	0	390	69	13

Appendix D. (Continued)

Anaerobic Organisms	Number of Strains	Clindamycin		Number of Strains	Moxifloxacin		Number of Strains	Metronidazole	
Percent susceptible (%S) and percent resistant (%R)[b]		%S	%R		%S	%R		%S	%R
Breakpoints, µg/mL		≤ 2	≥ 8		≤ 2	≥ 8		≤ 8	≥ 32
Prevotella spp.	29[c]	69[c]	28[c]	92	66	25	92	99	0
Fusobacterium spp.	75	77	21	75	68	23	75	95	5
Anaerobic gram-positive cocci[e]	1826	97	3	300	72	21	1692	100	0
C. acnes[f]	17[c]	53[c]	35[c]	114	95	4	18[c]	0[c]	100[c]
C. perfringens	425	83	12	23[c]	83[c]	9[c]	425	100	0
Clostridioides (formerly *Clostridium*) *difficile*[g]	1013	32	38	480	74	25	1343	100	0
Other *Clostridium* spp.	461	67	25	71	62	35	461	100	0

Abbreviation: MIC, minimal inhibitory concentration.

Footnotes

a. Data were generated from unique isolates from patient specimens submitted to Tufts Medical Center, Boston, Massachusetts; International Health Management Associates, Inc., Schaumburg, Illinois; R.M. Alden Research Laboratory, Culver City, California; Creighton University School of Medicine, Omaha, Nebraska; Mayo Clinic College of Medicine and Science, Rochester, Minnesota; and the Centers for Disease Control and Prevention, Atlanta, Georgia. All testing was performed by the agar dilution method. Information and analysis of previous versions of this table have been published.

b. Intermediate category is not shown but can be derived by subtraction of %S and %R for each antimicrobial agent from %100.

c. Calculated from fewer than the CLSI M39[1] recommendation of 30 isolates.

d. A dash (–) indicates that data were not available.

e. Anaerobic gram-positive cocci include *Peptococcus, Peptostreptococcus, Finegoldia, Peptoniphilus,* and *Anaerococcus* species.

f. 80 isolates of *C.* (formerly *P.*) *acnes* from two of the sites generated MIC values for rifampin ≤ 0.03 µg/mL using the agar dilution method. There are no interpretive breakpoints for this organism/antimicrobial agent combination.

g. *C.* (formerly *Clostridium*) *difficile* isolates are from an intestinal source; these results do not imply efficacy for intraluminal infections. Vancomycin MICs for isolates were < 4 µg/mL.

Appendix D. (Continued)

Reference for Appendix D

[1] CLSI. *Analysis and Presentation of Cumulative Antimicrobial Susceptibility Test Data*. 5th ed. CLSI guideline M39. Clinical and Laboratory Standards Institute; 2022.

Appendix E. Susceptible-Dose Dependent Interpretive Category

Abbreviations for Appendix E

AST	antimicrobial susceptibility testing
FDA	US Food and Drug Administration
MIC	minimal inhibitory concentration
QC	quality control
SDD	susceptible-dose dependent

Susceptible-dose dependent (SDD) is recommended instead of "intermediate" for several drug and organism combinations for which there are multiple approved or routinely used dosing options:

- Enterobacterales: cefepime, piperacillin, and piperacillin-tazobactam
- *Staphylococcus aureus:* ceftaroline
- *Enterococcus faecium:* daptomycin

SDD highlights the option of using higher doses or alternative dosage regimens by which to achieve a higher dose exposure for the treatment of infections caused by isolates when the minimal inhibitory concentration (MIC) or the zone diameter is in the SDD range.

What does SDD mean?

SDD is a category defined by a breakpoint that implies that susceptibility of an isolate depends on the dosage regimen that is used in the patient. To achieve levels that are likely to be clinically effective against isolates for which the susceptibility testing results (either MICs or zone diameters) are in the SDD category, it is necessary to use a dosage regimen (ie, higher doses, more frequent doses, or both) that results in higher drug exposure than that achieved with the dose that was used to establish the susceptible breakpoint. Consideration should be given to the maximum, literature-supported dosage regimens, because higher exposure gives the highest probability of adequate coverage of an SDD isolate. Table 2 Dosages lists the doses used when establishing SDD categories. The drug label should be consulted for recommended doses and adjustment for organ function.

NOTE: The concept of SDD has been included within the intermediate category definition for antimicrobial agents. However, this is often overlooked or not understood by clinicians and microbiologists when an intermediate result is reported. The SDD category may be assigned when doses well above those used to calculate the susceptible breakpoint are supported by the literature, widely used clinically, and/or approved and for which sufficient data to justify the designation exist and have been reviewed. When the intermediate category is used, its definition remains unchanged.

Appendix E. (Continued)

Why is SDD being used now?

- There is a growing need to refine susceptibility reporting to maximize clinicians' use of available drugs.
- Intermediate too often means "resistant" to clinicians because they do not appreciate the full definition of "intermediate."
- SDD is more specific and conveys what we know—a higher dose can be considered for isolates with MICs (or zones of inhibition) that fall in this interpretive category.
- SDD is already well established for use in antifungal susceptibility testing.
- Antibiotic stewardship programs, which emphasize dosage regimen and duration of therapy options, are increasing awareness of appropriate use of antibiotics. Personnel from these programs should be able to describe the significance to clinicians of an SDD result.

How should this change be implemented?

- Meet with the appropriate practitioners at your institution (eg, members of the antimicrobial stewardship team and other relevant institutional stakeholders) to explain SDD and determine a plan for implementation, if appropriate.
- Talk to the manufacturer of your antimicrobial susceptibility testing (AST) device to determine how to implement reporting SDD on your device.
  - **NOTE:** Because the US Food and Drug Administration (FDA) does not yet recognize the SDD interpretive category and commercial manufacturers must use FDA breakpoints, the manufacturer cannot adopt the CLSI SDD breakpoints. However, for most systems, you can manually change the breakpoints and implement, following a verification study.
- Work with your laboratory information system staff to report "SDD" or dose ("D") when MICs or zone diameters are in the SDD range. Some laboratory information systems may handle only a single character and use of "D" for "dose" may be appropriate. Ideally, this could be translated to SDD on the final patient report. Regardless of approach, make certain that SDD will be transmitted to the hospital information system and appropriately displayed on reports viewed by clinicians.
- Distribute user-specific educational materials to laboratory staff and clinicians receiving AST results from your laboratory. Examples of these materials can be found on the CLSI Subcommittee on Antimicrobial Susceptibility Testing webpage at www.clsi.org.

Additional Questions and Answers:

1. Q: Does CLSI recommend a comment to be reported with the new SDD breakpoints?

   A: If a laboratory chooses to report a comment explaining the SDD range, CLSI recommends the following: "The interpretive criterion for susceptible is based on a dosage regimen of [dose] (refer to Table 2 Dosages). The interpretive criterion for SDD is based on dosage regimens that result in higher antimicrobial exposure, either higher doses or more frequent doses, or both."

Appendix E. (Continued)

2. Q: Will all intermediate ranges become SDD?

   A: No, the SDD category will be implemented for drug and organism combinations only when there is sufficient evidence to suggest alternative approved dosage regimens may be appropriate for organisms that have MICs or zone diameters between the susceptible and resistant categories.

3. Q: Will SDD be applied to other antimicrobial agents?

   A: CLSI will examine the SDD category possibility for additional drug and organism combinations for which multiple dosing options exist and have been well studied.

4. Q: How do we perform a verification study before implementing the new breakpoints on our AST device?

   A: Guidelines for performance of such a verification study are available (see CLSI M52[1]).[2]

5. Q: Does SDD apply to all patients and specimen types (eg, pediatric, geriatric, immunosuppressed)?

   A: Yes, in terms of laboratory reporting. Clinicians must decide how to use an SDD result for a specific patient while considering all other clinical and physiological parameters for that patient.

6. Q: Is any special QC needed once the SDD breakpoints are implemented?

   A: No, currently recommended routine QC is sufficient.

7. Q: Will it be necessary to report SDD on proficiency testing survey samples?

   A: Sponsors of proficiency testing surveys are aware of the difficulties encountered by laboratories in implementing newer CLSI breakpoints. It is highly unlikely that there will be a mandate to report SDD in the near future, but it would be best to check with your proficiency testing survey provider.

8. Q: If we can implement the revised breakpoints but cannot facilitate reporting of SDD, can we report “intermediate” instead of SDD?

   A: A decision related to this question should be made following consultation with the antimicrobial stewardship team and other relevant institutional stakeholders.

9. Q: If we can implement the revised breakpoints but cannot facilitate reporting of SDD, can we report an MIC or zone diameter without an interpretation?

   A: A zone diameter should never be reported without an interpretation because there is a high risk of misinterpretation of this value, which poses patient safety issues. There is a lesser danger of reporting an MIC without an interpretation, but this should not be done without an accompanying qualifying comment. See answer to question 8, above.

Appendix E. (Continued)

10. Q: What does the dosing information that is given with breakpoints mean?

A: The evolving science of pharmacokinetics/pharmacodynamics has become increasingly important in recent years in determining MIC breakpoints. Recently approved susceptible or SDD breakpoints for a number of agents have been based on a specific dosage regimen(s); these dosage regimens are listed in Table 2 Dosages. Proper application of the breakpoints necessitates drug exposure at the site of infection that corresponds to or exceeds the expected systemic drug exposure, at the dose listed, in adult patients with normal renal function. This information should be shared with pharmacists, infectious diseases staff, and others making dosing recommendations for the institution.

References for Appendix E

[1] CLSI. *Verification of Commercial Microbial Identification and Antimicrobial Susceptibility Testing Systems.* 1st ed. CLSI guideline M52. Clinical and Laboratory Standards Institute; 2015.

[2] Patel JB, Sharp S, Novak-Weekley S. Verification of antimicrobial susceptibility testing methods: a practical approach. *Clin Microbiol Newslett.* 2013;35(13):103-109. doi:10.1016/j.clinmicnews.2013.06.001

Appendix F. Epidemiological Cutoff Values

Abbreviations for Appendix F

ECV	epidemiological cutoff value
MIC	minimal inhibitory concentration
NWT	non-wild-type
WT	wild-type

F1 CLSI Epidemiological Cutoff Value Additions/Revisions Since 2015

Antimicrobial Agent	Date of Addition/Revision (CLSI M100 edition)	Comment
Burkholderia cepacia Complex		
Ceftazidime **Levofloxacin** **Meropenem** **Minocycline** **Trimethoprim-sulfamethoxazole**	**January 2025 (M100-Ed35)**	**Developed in consideration of the breakpoints listed in M100 34th ed (2024) that were removed and archived.**
Anaerobes		
Vancomycin	January 2015 (M100-S25)	For use with *Cutibacterium* (formerly *Propionibacterium*) *acnes*.

F2 Defining Epidemiological Cutoff Values

F2.1 Definitions

epidemiological cutoff value (ECV) – the minimal inhibitory concentration (MIC) or zone diameter value that separates microbial populations into those with and without phenotypically detectable resistance (non-wild-type [NWT] or wild-type [WT], respectively). The ECV defines the highest MIC or smallest zone diameter for the WT population of isolates.

EXAMPLE:

Interpretive Category	MIC, µg/mL	Zone Diameter, mm
Wild-type[a]	≤ 4	≥ 20
Non-wild-type	≥ 8	≤ 19

Footnote

a. In the example above, the ECV is 4 µg/mL (MIC) and 20 mm (zone diameter).

Appendix F. (Continued)

- **wild-type (WT)** – an interpretive category defined by an ECV that describes the microbial population with no phenotypically detectable mechanisms of resistance or reduced susceptibility for the antimicrobial (antifungal) agent being evaluated.
- **non-wild-type (NWT)** – an interpretive category defined by an ECV that describes the microbial population with phenotypically detectable mechanisms of resistance and reduced susceptibility for the antimicrobial (antifungal) agent being evaluated.

F2.2 Epidemiological Cutoff Values vs Clinical Breakpoints

ECVs are based on *in vitro* data only, using MIC or zone diameter distributions. ECVs are not clinical breakpoints, and the clinical relevance of ECVs for a particular patient has not yet been identified or approved by CLSI or any regulatory agency. By contrast, clinical breakpoints are established using MIC distributions, pharmacokinetic/pharmacodynamic data, and clinical outcome data, when available (as described in CLSI M23[1]).

"Caution": Zone diameter (disk diffusion) and MIC values for which ECVs are defined are not to be interpreted or reported as susceptible, intermediate, or resistant but rather as WT or NWT. The ECVs should not be used as clinical breakpoints.

F2.3 Establishing Epidemiological Cutoff Values

ECVs are determined by collecting and merging MIC distribution data obtained by testing microbes from a variety of sources and then applying statistical techniques for estimating the MIC at the upper end of the WT distribution. Subsequently, corresponding zone diameter data from disk diffusion testing are examined and a disk diffusion ECV is determined, when appropriate. To ensure reliability, ECVs are estimated while accounting for both biological (strain-to-strain) variation and MIC/disk assay variation within and between laboratories. They are based on the assumption that the WT distribution of a particular antimicrobial agent–organism combination does not vary geographically or over time.

Several conditions must be fulfilled to generate reliable ECVs. The most important are:

- An ECV can be determined only within a single species for a single agent because of the genetic diversity between species within a genus.
- All MIC values included in the dataset must have been determined using a standard reference method (eg, the CLSI MIC broth dilution method as described in CLSI M07,[2] which is also the method outlined in an international reference standard[3]). Similarly, the standard reference disk diffusion method as described in CLSI M02[4] must be used when zone diameter ECVs are defined.
- Data must be sourced from at least three separate laboratories and at least 100 unique isolates must be included in the merged dataset.
- MIC values contributed from an individual laboratory dataset should be "on scale" (ie, the MIC is not below the lowest or above the highest concentration tested), whenever possible. This is particularly important for MICs of the presumptive WT strains. Before merging data from individual laboratories, the MIC distribution from each laboratory must be inspected, and if the lowest concentration tested is also the mode, the data must be excluded.
  - Once acceptable data are merged, there are several methods that can be used to estimate the ECV.

Appendix F. (Continued)

- Visual inspection is the simplest method and is generally acceptable for MIC distributions when there is clear separation of WT and NWT strains. When there is obvious overlap between WT and NWT strains, visual inspection is too subjective to set a reliable ECV.
- Statistical methods are preferred because they remove potential observer bias from the estimation. The two most widely referenced statistical methods are those described by Turnidge et al.[5] and by Kronvall.[6]

– Establishment of ECVs from MIC distributions may be supplemented with molecular tests for known resistance genes. The detection of a resistance gene per se in strains with MICs at or below the ECV does not necessarily contradict the choice of ECV, unless it can be accompanied by evidence that the gene is being expressed. In such cases, the ECV may need to be reassessed.

F2.4 Epidemiological Cutoff Value Use by the Medical Microbiology Laboratory

The need for testing and interpreting drug and organism combinations with an ECV but no clinical breakpoint must be discussed with appropriate clinical specialists (eg, antibiotic stewardship, infectious diseases, and pharmacy). While ECVs do not predict clinical outcome, laboratories may consider noting WT or NWT MIC (or zone diameter) interpretations on laboratory reports. Many physicians may choose not to consider using antimicrobial agents with an NWT interpretation, if other therapeutic options are available. However, it is critical that laboratories refrain from reporting WT as susceptible, or NWT as resistant, as there are insufficient clinical data to support this practice. ECVs may be used to signal the emergence of resistance, although this application for ECVs is best suited to public health laboratories and surveillance studies.

F3 Epidemiological Cutoff Value Tables

"Caution": Zone diameter (disk diffusion) and MIC values for which ECVs are defined are not to be interpreted or reported as susceptible, intermediate, or resistant but rather as WT or NWT. The ECVs should not be used as clinical breakpoints.

Table F1. ECVs for *Burkholderia cepacia* Complex[a]

Antimicrobial Agent	Interpretive Category and MIC, µg/mL		Comment
	WT[b,c]	NWT	
Ceftazidime	≤ 16	≥ 32	
Levofloxacin	≤ 8	≥ 16	
Meropenem	≤ 16	≥ 32	
Minocycline	≤ 8	≥ 16	
Trimethoprim-sulfamethoxazole	≤ 2	≥ 4	

Abbreviations: ECV, epidemiological cutoff value; MIC, minimal inhibitory concentration; NWT, non-wild-type; WT, wild-type.

Appendix F. (Continued)

Footnotes

a. Insufficient data were available to establish ECVs for individual species within the *B. cepacia* complex. Although more than 50% of the data were contributed by a single laboratory for minocycline and trimethoprim-sulfamethoxazole, the data were not weighted before pooling and analysis. The ECVs are under review and will be updated if appropriate.

b. The ECV is the highest MIC that defines the WT population of isolates (eg, the ECV for ceftazidime is 16 µg/mL and the WT population is ≤ 16 µg/mL).

c. The ECVs for ceftazidime, levofloxacin, meropenem, and minocycline are above MICs typically achievable by routine antimicrobial dosing for similar organisms and are higher than the archived susceptible breakpoints (8, 2, 4, and 4 µg/mL, respectively).

ECVs listed in Table F2 are applicable only to the species indicated. Currently, there are insufficient data to support their use with other species.

Table F2. ECVs for Specific Anaerobic Species

Antimicrobial Agent	Interpretive Category and MIC, µg/mL		Comment
	WT[a]	NWT	
Vancomycin	≤ 2	≥ 4	For use with *C.* (formerly *P.*) *acnes*[7-10] and *Clostridioides* (formerly *Clostridium*) *difficile*.[11-13]

Abbreviations: ECV, epidemiological cutoff value; MIC, minimal inhibitory concentration; NWT, non-wild-type; WT, wild-type.

Footnote

a. The ECV is the highest MIC that defines the WT population of isolates (eg, the ECV for vancomycin is 2 µg/mL and the WT population is ≤ 2 µg/mL).

NOTE: Information in boldface type is new or modified since the previous edition.

References for Appendix F

[1] CLSI. *Development of* In Vitro *Susceptibility Test Methods, Breakpoints, and Quality Control Parameters.* 6th ed. CLSI guideline M23. Clinical and Laboratory Standards Institute; 2023.

[2] CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically.* 12th ed. CLSI standard M07. Clinical and Laboratory Standards Institute; 2024.

[3] ISO. *Susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility test devices – Part 1: Broth micro-dilution reference method for testing the in vitro activity of antimicrobial agents against rapidly growing aerobic bacteria involved in infectious diseases.* ISO 20776-1. International Organization for Standardization; 2019.

Appendix F. (Continued)

[4] CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests*. 14th ed. CLSI standard M02. Clinical and Laboratory Standards Institute; 2024.

[5] Turnidge J, Kahlmeter G, Kronvall G. Statistical characterisation of bacterial wild-type MIC value distributions and the determination of epidemiological cut-off values. *Clin Microbiol Infect*. 2006;12(5):418-425. doi:10.1111/j.1469-0691.2006.01377.x

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[12] Goldstein EJC, Citron DM, Tyrrell KL, Merriam CV. Comparative *in vitro* activities of SMT19969, a new antimicrobial agent, against *Clostridium difficile* and 350 gram-positive and gram-negative aerobic and anaerobic intestinal flora isolates. *Antimicrob Agents Chemother*. 2013;57(10):4872-4876. doi:10.1128/AAC.01136-13

[13] Goldstein EJC, Babakhani F, Citron DM. Antimicrobial activities of fidaxomicin. *Clin Infect Dis*. 2012;55(suppl 2):S143-S148. doi:10.1093/cid/cis339

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Appendix G. Using Molecular Assays for Resistance Detection

Antimicrobial resistance and susceptibility are complex, and current *in vitro* methods have been developed to predict a microorganism's response to antibacterial therapy *in vivo*. Standardized phenotypic methods have evolved over many decades, but faster and potentially more reliable nucleic acid– and protein-based methods have been recently developed to detect antimicrobial resistance. The current challenge for medical laboratories is to integrate molecular assays for antimicrobial resistance determinants with conventional antimicrobial susceptibility testing procedures, sometimes despite an incomplete understanding of test limitations.

The tables in this appendix provide a practical approach for testing and reporting results among medical laboratories that routinely use molecular techniques (with or without a phenotypic test) for detecting antimicrobial resistance. Antimicrobial resistance is genetically complex and based on available data. Molecular methods are often used as a screening tool (eg, methicillin [oxacillin]-resistant *Staphylococcus aureus* from nasal swabs) or as a rapid adjunct to traditional phenotypic methods (eg, KPC from instrument-flagged blood culture bottles). Interpretation necessitates critical thinking and an understanding of the dynamics between detecting "resistance" determinants and testing phenotypic "susceptibility." Detecting a resistance marker does not necessarily predict therapeutic failure of antimicrobial agents. The gene may be nonfunctional or expressed at clinically insignificant levels. Conversely, the absence of the genetic marker does not necessarily indicate susceptibility, because technical issues may interfere with detection (eg, inhibition of amplification, emergence of genetic variants). In some cases, a molecular approach may be superior to traditional phenotypic methods, such as in the case of low *in vitro* expression, heteroresistance, or poor growth masking higher minimal inhibitory concentrations. Overall, laboratorians should attempt to apply a consistent approach to molecular-based methods and aim to resolve discordant results with repeat or supplementary testing, by referral to a reference laboratory or by reporting both results in accordance with institutional policies.

As understanding of the molecular mechanisms of antimicrobial resistance continues to develop, more sophisticated approaches to molecular detection of antimicrobial resistance in the medical microbiology laboratory will undoubtedly emerge. The following tables will be updated as needed to ensure the provision of relevant guidance as methods evolve.

Appendix G. (Continued)

Table G1. Strategies for Reporting Methicillin (Oxacillin) Results When Using Molecular and Phenotypic AST Methods for *S. aureus*

Indication	Resistance Mechanism(s)	Methods	Specimen Types	Results		Suggestions for Resolution	Consider reporting as[a]:	Comments[b]
				Resistance Mechanism(s) Detected	Phenotypic AST (if tested)			
Detecting methicillin (oxacillin) resistance in *S. aureus*	PBP2a	Latex agglutination, immunochromatography	Colony	PBP2a positive	Cefoxitin R	N/A	Methicillin (oxacillin) R	1
				PBP2a negative	Cefoxitin S	N/A	Methicillin (oxacillin) S	1
				PBP2a positive	Cefoxitin S	Confirm isolate identification, repeat latex agglutination and AST, and consider *mecA* colony NAAT, if available.	If discrepancy is not resolved by suggested testing, report as methicillin (oxacillin) R.	1–2
				PBP2a negative	Cefoxitin R	Confirm isolate identification, repeat latex agglutination and AST, and consider *mecA* colony NAAT, if available.	If discrepancy is not resolved by suggested testing, report as methicillin (oxacillin) R.	1
	mecA	NAAT, microarray hybridization, ISH	Colony, blood culture broth, surveillance specimen	*mecA* detected	Cefoxitin R	N/A	If tested, report phenotypic result as found (methicillin [oxacillin] R) and consider reporting molecular result per institutional protocol.	3–6
				mecA not detected	Cefoxitin S	N/A	If tested, report phenotypic result as found (methicillin [oxacillin] S) and consider reporting molecular result per institutional protocol.	3–6
				mecA detected	Cefoxitin S	Confirm isolate identification, repeat AST, and repeat or perform *mecA* colony NAAT, if available. If mixed specimen, test isolates individually.	If discrepancy is not resolved by suggested testing, report as methicillin (oxacillin) R.	2–5, 8–9
				mecA not detected	Cefoxitin R	Confirm isolate identification, repeat AST, and repeat or perform *mecA* colony NAAT, if available. If mixed specimen, test isolates individually.	If discrepancy is not resolved by suggested testing, report as methicillin (oxacillin) R.	3, 7

Appendix G. (Continued)

Table G1. (Continued)

Indication	Resistance Mechanism(s)	Methods	Specimen Types	Results		Suggestions for Resolution	Consider reporting as[a]:	Comments[b]
				Resistance Mechanism(s) Detected	Phenotypic AST (if tested)			
Detecting methicillin (oxacillin) resistance in *S. aureus* (Continued)	SCC*mec*- *orfX* functional regions only	NAAT	Blood culture broth, surveillance specimen	SCC*mec* detected	Cefoxitin R	N/A	If tested, report phenotypic result as found (methicillin [oxacillin] R) and consider reporting molecular result per institutional protocol.	3–6
				SCC*mec* not detected	Cefoxitin S	N/A	If tested, report phenotypic result as found (methicillin [oxacillin] S) and consider reporting molecular result per institutional protocol.	3–6
				SCC*mec* detected	Cefoxitin S	Confirm isolate identification, repeat AST and consider *mecA* colony NAAT, if available. If mixed culture, test isolates individually.	If discrepancy is not resolved by suggested testing, report as methicillin (oxacillin) R.	2, 10
				SCC*mec* not detected	Cefoxitin R	Confirm isolate identification, repeat AST and consider *mecA* colony NAAT, if available. If mixed culture, test isolates individually.	If discrepancy is not resolved by suggested testing, report as methicillin (oxacillin) R.	7, 11

Appendix G. (Continued)

Table G1. (Continued)

Indication	Resistance Mechanism(s)	Methods	Specimen Types	Results		Suggestions for Resolution	Consider reporting as[a]:	Comments[b]
				Resistance Mechanism(s) Detected	Phenotypic AST (if tested)			
Detecting methicillin (oxacillin) resistance in *S. aureus* (Continued)	SCC*mec-orfX* junctional regions and *mecA* and/or other targets	NAAT	Blood culture broth, surveillance specimen	SCC*mec* AND *mecA* or other target detected	Cefoxitin R	N/A	If tested, report phenotypic result as found (methicillin [oxacillin] R) and consider reporting molecular result per institutional protocol.	3–6
				SCC*mec* AND *mecA* or other target not detected	Cefoxitin S	N/A	If tested, report phenotypic result as found (methicillin [oxacillin] S) and consider reporting molecular result per institutional protocol.	3–6
				SCC*mec* AND *mecA* or other target detected	Cefoxitin S	Confirm isolate identification, repeat AST and consider *mecA* colony NAAT, if available. If mixed culture, test isolates individually.	If discrepancy is not resolved by suggested testing, report as methicillin (oxacillin) R.	2
				SCC*mec* AND *mecA* or other target not detected	Cefoxitin R	Confirm isolate identification, repeat AST and consider *mecA* colony NAAT, if available. If mixed culture, test isolates individually.	If discrepancy is not resolved by suggested testing, report as methicillin (oxacillin) R.	3, 11

Abbreviations: AST, antimicrobial susceptibility testing; ISH, *in situ* hybridization; MRSA, methicillin (oxacillin)-resistant *Staphylococcus aureus;* MSSA, methicillin (oxacillin)-susceptible *Staphylococcus aureus*; N/A, not applicable; NAAT, nucleic acid amplification test; PBP2a, penicillin-binding protein 2a; PCR, polymerase chain reaction; R, resistant; S, susceptible.

Appendix G. (Continued)

Comments

(1) False-positive and false-negative PBP2a latex bead agglutination results have been observed.[1]

(2) Rare *mecA*-positive *S. aureus* isolates will test susceptible to cefoxitin.[2,3]

(3) *mecC* or *mecA* variant gene-mediated methicillin (oxacillin) resistance may not be detected by *mecA* PCR.[4,5]

(4) The simultaneous presence of *mecA*-positive *Staphylococcus* spp. (other than *S. aureus*) and MSSA may result in false-positive MRSA molecular results.[6,7]

(5) Strains harboring unstable SCC*mec* insertions may lose *mecA* during culture.[8]

(6) Compared with culture, the sensitivity of molecular methods may be higher, while the specificity may be lower.

(7) Occasional false-negative *mecA* results have been reported for direct blood culture molecular assays.[9]

(8) For ISH assays with a cefoxitin induction step, false-positive *mecA* results should be rare.[10]

(9) In polymicrobial cultures, the presence of *mecA* cannot be attributed to a specific isolate.

(10) Strains harboring an SCC*mec* remnant lacking the *mecA* gene (*mecA* dropout) or mutant *mecA* allele may test positive in assays that target only SCC-*mec-orfX* junctional regions. Laboratories using molecular tests that detect only SCC-*mec-orfX* junctional region targets may consider adding a disclaimer to the report stating the proportion of false-positive results related to *mecA* dropouts observed in isolates from the patient population served.[11]

(11) Multiple SCC*mec* types exist; depending on the design of the assay, some SCC*mec* variants may not be detected.[12]

Footnotes

a. Isolates that test as methicillin resistant are also oxacillin resistant, and the term "methicillin R" is synonymous with "oxacillin R."

b. In addition to the specific possibilities listed in the comments, genotype and/or phenotype discrepancies could arise as a consequence of suboptimal sampling, mixed cultures, emergence of new genotypes or mutations, and/or wild-type reversions of resistance targets.

Appendix G. (Continued)

Table G2. Strategies for Reporting Vancomycin Results When Using Molecular and Phenotypic AST Methods for *Enterococcus* spp.

Indication	Resistance Mechanism(s)	Methods	Specimen Types	Results		Suggestions for Resolution	Report as:	Comments[a]
				Resistance Mechanism(s) Detected	Phenotypic AST (if tested)			
Detection of VRE	*vanA* *vanB*	NAAT or array hybridization technology	Blood culture broth or surveillance cultures	*vanA* and/or *vanB* detected	Vancomycin R	N/A	Report phenotypic result as found (if available); consider reporting presence of molecular target per institutional protocol.	1–3
				vanA and/or *vanB* not detected	Vancomycin S	N/A	Report phenotypic result as found (if available); consider reporting presence of molecular target per institutional protocol.	
				vanA and/or *vanB* detected	Vancomycin S	Confirm isolate identification to species level (eg, *E. faecalis*) and repeat AST. If mixed culture, test isolates individually.	If discrepancy is not resolved by suggested testing, report as vancomycin R.	1–3
				vanA and/or *vanB* not detected	Vancomycin R	Confirm isolate identification to species level (eg, *E. faecalis*) and repeat AST. If mixed culture, test isolates individually.	If discrepancy is not resolved by suggested testing, report as vancomycin R.	4

Appendix G. (Continued)

Table G2. (Continued)

Indication	Resistance Mechanism(s)	Methods	Specimen Types	Results		Suggestions for Resolution	Report as:	Comments[a]
				Resistance Mechanism(s) Detected	Phenotypic AST (if tested)			
Detection of VRE (Continued)	*vanA*	NAAT	Surveillance cultures	*vanA* detected	Vancomycin R	N/A	Report phenotypic result as found (if available); consider reporting presence of molecular target per institutional protocol.	1–2
				vanA not detected	Vancomycin S	N/A	Report phenotypic result as found (if available); consider reporting presence of molecular target per institutional protocol.	5
				vanA detected	Vancomycin S	Confirm isolate identification to species level (eg, *E. faecalis*) and repeat AST. If mixed culture, test isolates individually.	If the discrepancy is not resolved by suggested testing, report as vancomycin R.	1–2
				vanA not detected	Vancomycin R	Confirm isolate identification to species level (eg, *E. faecalis*) and repeat AST. If mixed culture, test isolates individually.	If the discrepancy is not resolved by suggested testing, report as vancomycin R.	4–5

Abbreviations: AST, antimicrobial susceptibility testing; N/A, not applicable; NAAT, nucleic acid amplification test; R, resistant; S, susceptible; VRE, vancomycin-resistant enterococci.

Appendix G. (Continued)

Comments

(1) *vanA* may be present in nonenterococcal species.[13]

(2) Vancomycin-variable *E. faecium* isolates have been isolated in Canada. They carry wild-type *vanA* but initially test as vancomycin susceptible with a culture-based method. They can convert to a resistant phenotype during vancomycin treatment.[14,15]

(3) The *vanB* gene has been found in several commensal nonenterococcal bacteria, which may lead to misclassification of vancomycin-susceptible enterococci as resistant in surveillance cultures containing mixed bacterial species.[16]

(4) Constitutive low-level vancomycin resistance can be detected phenotypically (2–32 µg/mL) from the presence of *vanC*, an intrinsic resistance characteristic of *E. gallinarum* (*vanC1*) and *E. casseliflavus* (*vanC2–C4*).[17]

(5) Targeting *vanA* only may miss regional *vanB*-carrying VRE.[18]

Footnote

a. In addition to the specific possibilities referenced in the comments, genotype and/or phenotype discrepancies could arise as a consequence of suboptimal sampling, mixed cultures, emergence of new genotypes, or mutations and/or wild-type reversions of resistance targets.

Appendix G. (Continued)

Table G3. Reporting Results From ESBL Resistance and Carbapenemase Molecular Tests for Enterobacterales

Indication	Resistance Mechanism(s)	Methods	Specimen Types	Results		Suggestions for Resolution	Report as:	Comments[a]
				Resistance Mechanism(s) Detected	Phenotypic AST (if tested)			
Detection of ESBL resistance in Enterobacterales (in an isolate susceptible to all carbapenems)	ESBL type CTX-M, SHV, TEM	NAAT, microarray	Colony, blood culture	Detection of any ESBL target	R to all third- and fourth-generation cephalosporins tested (eg, ceftriaxone R, cefotaxime R, ceftazidime R, cefepime R)	N/A	Report phenotypic results as found (if available); consider reporting presence of molecular target per institutional protocol.	1–12
				Detection of any ESBL target	S to all third- and fourth-generation cephalosporins tested (eg, ceftriaxone S, cefotaxime S, ceftazidime S, cefepime S)	Repeat molecular and phenotypic tests. If blood culture, check for mixed culture. If mixed, test isolates individually and report phenotypic results as found.	If the discrepancy is not resolved, repeat AST should be performed using a reference method, and the conflicting genotypic and phenotypic testing results should both be reported.	1–12
				Detection of *CTX-M* ESBL target	Variable resistance to third- and fourth-generation cephalosporins (eg, ceftriaxone R, cefotaxime R, ceftazidime R or S, cefepime R or S)	Expected phenotype for some *CTX-M* strains. Check cefepime using a reference method if S.	Report phenotypic results as found, including reference cefepime result; consider reporting presence of molecular target per institutional protocol.	1–12
				Detection of *TEM* or *SHV* ESBL target	Variable resistance to third- and fourth-generation cephalosporins (eg, ceftriaxone R or S, cefotaxime R or S, ceftazidime R or S, cefepime R or S)	Expected phenotype for some *TEM/SHV* strains. Check cefepime using a reference method if S.	Report phenotypic results as found, including reference cefepime result; consider reporting presence of molecular target per institutional protocol.	1–12

Appendix G. (Continued)

Table G3. (Continued)

Indication	Resistance Mechanism(s)	Methods	Specimen Types	Results		Suggestions for Resolution	Report as:	Comments[a]
				Resistance Mechanism(s) Detected	Phenotypic AST (if tested)			
Detection of ESBL resistance in Enterobacterales (in an isolate susceptible to all carbapenems) (Continued)	ESBL type CTX-M, SHV, TEM	NAAT, microarray	Colony, blood culture	No detection of ESBL targets	Resistance to third-generation cephalosporins and variable resistance to fourth-generation cephalosporins (eg, ceftriaxone R, cefotaxime R, ceftazidime R, cefepime R or S)	Likely non-tested broad spectrum β-lactamase (eg, AmpC, carbapenemase, or other ESBL); consider repeating molecular tests and checking cefepime using reference method if S.	Report phenotypic results as found, including reference cefepime result if tested.	1–12
Detection of carbapenem resistance in Enterobacterales	KPC, OXA-48-like, VIM, NDM, or IMP Or Phenotypic evidence of a carbapenemase (eg, mCIM or Carba NP positive)	NAAT, microarray	Colony, blood culture	Detection of any tested carbapenemase target	Resistance to all carbapenems (eg, meropenem R, imipenem R, doripenem R, ertapenem R)	N/A	Report phenotypic results as found (if available); consider reporting presence of molecular target per institutional protocol.	1–4, 12–14
				Detection of any tested carbapenemase target	Susceptible to all carbapenems except ertapenem (variable) (eg, meropenem S, imipenem S, doripenem S, ertapenem R or S)	Repeat molecular and phenotypic tests. If blood culture, check for mixed culture. If mixed, test isolates individually and report phenotypic results as found; consider a phenotypic test for carbapenemase activity (such as Carba NP or mCIM).	If the discrepancy is not resolved, repeat AST should be performed using a reference method and the conflicting genotypic and phenotypic testing results should both be reported along with a comment advising caution; current clinical and laboratory evidence is insufficient to conclude whether carbapenem monotherapy of carbapenemase-carrying strains with an MIC in the S range will be effective, or whether the molecular assays are completely accurate.	1–4, 12–15

Appendix G. (Continued)

Table G3. (Continued)

Indication	Resistance Mechanism(s)	Methods	Specimen Types	Results		Suggestions for Resolution	Report as:	Comments[a]
				Resistance Mechanism(s) Detected	Phenotypic AST (if tested)			
Detection of carbapenem resistance in Enterobacterales (Continued)	KPC, OXA-48-like, VIM, NDM, or IMP Or Phenotypic evidence of a carbapenemase (eg, mCIM or Carba NP positive)	NAAT, microarray, phenotypic methods such as those described in Tables 3B and 3C	Colony, blood culture	Detection of any tested carbapenemase target(s) or phenotypic detection of carbapenemase production	Susceptibility to third-generation cephalosporins but intermediate or resistant to at least one carbapenem tested	Repeat resistance mechanism test(s) and AST.	If the discrepancy is not resolved, repeat AST should be performed using a reference method, and the conflicting genotypic and phenotypic testing results should both be reported along with a comment advising caution: "Current clinical and laboratory evidence is insufficient to conclude whether cephalosporin therapy of carbapenemase-carrying strains with an MIC in the S range will be effective."	1–4, 12–14
				Detection of any tested carbapenemase target(s) or phenotypic detection of carbapenemase production	Susceptibility (S or SDD) to cefepime	If this is an unexpected phenotype in your institution, consider repeating resistance mechanism test(s) and AST.	If the discrepancy is not resolved, cefepime should be suppressed or reported as R. **NOTE:** Current evidence suggests cefepime therapy may not be effective against carbapenemase-producing strains. Most of these data are based on studies investigating KPC-producing CREs.	1–4, 12–14

Appendix G. (Continued)

Table G3. (Continued)

Indication	Resistance Mechanism(s)	Methods	Specimen Types	Results		Suggestions for Resolution	Report as:	Comments[a]
				Resistance Mechanism(s) Detected	Phenotypic AST (if tested)			
Detection of carbapenem resistance in Enterobacterales (Continued)	KPC, OXA-48-like, VIM, NDM, or IMP Or Phenotypic evidence of a carbapenemase (eg, mCIM or Carba NP positive)	NAAT, microarray	Colony, blood culture	No detection of tested carbapenemase targets	Susceptible to all carbapenems except ertapenem (eg, meropenem S, imipenem S, doripenem S, ertapenem R)	Likely ESBL/AmpC and porin alteration, especially for *Enterobacter* spp.; consider a phenotypic test for carbapenemase activity (eg, Carba NP or mCIM); carbapenemase unlikely if negative, although rare carbapenemases (eg, GES-types, are still possible).	If carbapenemase activity is detected, repeat AST should be performed using a reference method, and the conflicting genotypic and phenotypic testing results should both be reported along with a comment advising caution; current clinical and laboratory evidence is insufficient to conclude whether carbapenem monotherapy of carbapenemase-carrying strains with an MIC in the susceptible range will be effective or whether the molecular assays are completely accurate. Otherwise, report phenotypic results as found.	1–4, 12–15

Appendix G. (Continued)

Table G3. (Continued)

Indication	Resistance Mechanism(s)	Methods	Specimen Types	Results		Suggestions for Resolution	Report as:	Comments[a]
				Resistance Mechanism(s) Detected	Phenotypic AST (if tested)			
Detection of carbapenem resistance in Enterobacterales (Continued)	KPC, OXA-48-like, VIM, NDM, or IMP Or Phenotypic evidence of a carbapenemase (eg, mCIM or Carba NP positive)	NAAT, microarray	Colony, blood culture	No detection of tested carbapenemase targets	Resistance to any carbapenems except ertapenem (eg, meropenem R, imipenem R, doripenem R, ertapenem R or S)	Possible other carbapenemase. If blood culture, check for mixed culture. If mixed, test isolates individually and report as found; consider repeating molecular and AST and performing a phenotypic test for carbapenemase activity (eg, Carba NP or mCIM).	If carbapenemase activity is detected, repeat AST should be performed using a reference method, and the conflicting genotypic and phenotypic testing results should both be reported along with a comment advising caution; current clinical and laboratory evidence is insufficient to conclude whether carbapenem monotherapy of carbapenemase-carrying strains with an MIC in the S range will be effective or whether the molecular assays are completely accurate. Otherwise, report phenotypic results as found.	1–4, 12–16

Abbreviations: AST, antimicrobial susceptibility testing; Carba NP, carbapenemase Nordmann-Poirel; CRE, carbapenem-resistant Enterobacterales; ESBL, extended-spectrum β-lactamase; mCIM, modified carbapenem inactivation method; MIC, minimal inhibitory concentration; N/A, not applicable; NAAT, nucleic acid amplification test; R, resistant; S, susceptible; SDD, susceptible-dose dependent.

Appendix G. (Continued)

Comments

(1) Multiple β-lactamases may be carried by individual bacterial isolates. Most carbapenemase-producing bacteria are resistant to third- and fourth-generation cephalosporins, although bacteria producing some carbapenemase enzymes (eg, OXA-48 and SME) may not test resistant unless they co-produce an ESBL or AmpC β-lactamase.

(2) Molecular assays can detect the presence of specific β-lactamase genes but cannot exclude the presence of other β-lactamase genes or resistance mechanisms, or novel variants with changes in primer or probe annealing sites. Therefore, phenotypic resistance should always be reported.

(3) Isolates with phenotypic susceptibility despite the presence of a resistance determinant may indicate the potential for resistance to emerge during therapy.

(4) These are provisional guidelines based on general principles; however, the performance characteristics of many individual research use–only assays are presently unknown.

(5) Susceptibility of TEM/SHV-carrying strains to β-lactam combinations is variable.

(6) Susceptibility of ESBL-carrying strains to cefepime is variable.

(7) Susceptibility of ESBL-carrying strains to β-lactam combination agents is variable.

(8) Some strains carrying CTX-M ESBLs remain susceptible to ceftazidime.

(9) Some strains carrying TEM/SHV-derived ESBLs remain susceptible to cefotaxime and ceftriaxone.

(10) Some molecular assays for AmpC may not reliably distinguish between chromosomal and plasmid-encoded genes in some bacterial species.

(11) Most strains with derepressed AmpC expression remain susceptible to cefepime.

(12) These recommendations are based on cephalosporin and carbapenem breakpoints in CLSI M100.

(13) The susceptibility to other carbapenems of ertapenem-resistant strains with ESBL or AmpC enzymes and reduced porin expression that do not contain carbapenemase genes or express carbapenemase activity may be reported as measured in phenotypic susceptibility assays.

(14) Rapid tests for carbapenemase activity (eg, Carba NP) may not detect OXA-48-like and some other carbapenemases.

(15) Caution is advised. Current clinical evidence is insufficient to conclude whether carbapenem monotherapy of carbapenemase-carrying strains with an MIC in the susceptible range will be effective.

(16) Some isolates of Enterobacterales, in particular but not exclusively *Morganella* spp., *Proteus* spp., and *Providencia* spp., may exhibit intrinsic low-level resistance to imipenem on a non–carbapenemase-mediated basis.

Appendix G. (Continued)

Footnote

a. In addition to the specific possibilities listed in the comments, genotype and/or phenotype discrepancies could arise as a consequence of mixed cultures, emergence of new genotypes, or mutations and/or wild-type reversions of resistance targets.

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Appendix G. (Continued)

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Appendix H. Modifications of the Minimal Inhibitory Concentration Method for Testing Select Antimicrobial Agents

Abbreviations for Appendix H

CAMHB-HSD	**cation-adjusted Mueller-Hinton broth supplemented with horse serum (25% v/v) and 0.5 mM DL-dithiothreitol (pH 7.2–7.4)**
ID-CAMHB	**iron-depleted cation-adjusted Mueller-Hinton broth**
MIC	**minimal inhibitory concentration**
pH	**negative logarithm of hydrogen ion concentration**

NOTE 1: Modifications to the CLSI reference broth microdilution minimal inhibitory concentration (MIC) method (see CLSI M07[1]) are required for testing certain antimicrobial agents.

NOTE 2: Appendix H, sections H1 and H2 describe the modifications required to test cefiderocol (Appendix H, section H1) and exebacase (Appendix H, section H2), including preparation of stock solutions, supplements, modified Mueller-Hinton broth, incubation conditions, and end point determination, as applicable.

NOTE 3: Information in boldface type is new or modified since the previous edition.

H1 Cefiderocol Broth Preparation and Reading Broth Microdilution Minimal Inhibitory Concentration End Points

H1.1 Supplements

H1.1.1 Calcium and Magnesium Stock Solutions

Refer to CLSI M07[1] for cation stock solution preparation.

H1.1.2 Zinc Stock Solution

The steps for preparing zinc stock solution are listed below.

Step	Action	Comments
1	Dissolve 0.29 g $ZnSO_4 \cdot 7H_2O$ in 100 mL deionized water.	This solution contains 0.65 mg Zn^{2+}/mL (10 mmol Zn^{2+}/mL).
		Verify that the deionized water has an iron content of ≤ 0.03 mg/L.
2	Sterilize the solution by membrane filtration.	
3	Store the solution at 15 to 25°C in a sterile single-use plastic container.	Previously used glass containers should be avoided to prevent inadvertent iron contamination.

Abbreviations: H_2O, water; $ZnSO_4$, zinc sulfate.

Appendix H. (Continued)

H1.2 Iron-Depleted Cation-Adjusted Mueller-Hinton Broth[a]

The steps for preparing iron-depleted cation-adjusted Mueller-Hinton broth (ID-CAMHB) are listed below.[2]

Step	Action	Comments
1	Prepare the CAMHB.	Follow manufacturer's instructions.
2	Autoclave the media and let cool to room temperature.	
3	Add 100 g chelating resin to 1 L autoclaved CAMHB.[2]	Removes polyvalent metal cations in the medium- to low-level concentrations (range, 0–0.18 mg/L).[2]
4	Stir the solution at room temperature for approximately 6 h using a magnetic stir bar.	
5	Filter the solution using a 0.2-µm filter.	Removes the resin. It is recommended that testing for residual iron levels of the filtrate should be conducted at this step to confirm that the iron content does not exceed 0.03 mg/L. Residual iron content can be measured with a commercial iron detection kit capable of detecting low levels of iron (0.02 mg/L). If iron levels exceed 0.03 mg/L, restart the procedure at the chelation step 3 above.
6	Check the pH to determine whether it is 7.3 ± 0.1.	If the pH is above 7.4, adjust it using 1 or 6 N HCl (use of 6 N HCl will minimize the volume required to adjust the pH). If the pH is below 7.2, use 2.5 N NaOH.
7	Add the cation to achieve final concentrations in the following ranges: • Ca^{2+} 20–25 mg/L • Mg^{2+} 10–12.5 mg/L • Zn^{2+} 0.5–1.0 mg/L	The final concentration of iron in ID-CAMHB prepared using this method should be ≤ 0.03 mg/L. Refer to CLSI M07[1] for calculating the amount of Ca^{2+}, Mg^{2+}, and the table below for calculating the amount of Zn^{2+} needed.
8	Check the pH to determine whether it is 7.3 ± 0.1.	If the pH exceeds 7.4, adjust it using 1 or 6 N HCl (use of 6 N HCl will minimize the volume required to adjust the pH). If the pH is below 7.2, use 2.5 N NaOH.
9	Filter the final product using a 0.2-µm filter.	
10	Store the media at 4 to 8°C for up to 2 mo.	

Abbreviations: CAMHB, cation-adjusted Mueller-Hinton broth; h, hour(s); HCl, hydrochloric acid; ID-CAMHB, iron-depleted cation-adjusted Mueller-Hinton broth; mo, month(s); NaOH, sodium hydroxide; pH, negative logarithm of hydrogen ion concentration.

Appendix H. (Continued)

Example for adding Zn^{2+} back to cation-adjusted Mueller-Hinton broth that contains below-detectable concentrations (< 0.0001 mg/L) of Zn^{2+} after chelation in step 3[2]:

Step	Action	Comments
1	Calculate the amount of Zn^{2+} needed using this formula: Final amount needed – amount in medium = amount to be added	For Zn^{2+}, the final amount needed is 0.5–1 mg/L. 1 mg/L – 0 mg/L = 1 mg/L
2	Add 1.54 mL Zn^{2+} stock per L (1.54 mL for each 1 mg/L).	C = concentration, V = volume $C_1 \cdot V_1$ = desired $C_2 \cdot$ final V_2 0.65 mg/mL $Zn^{2+} \cdot V_1$ = 1 mg Zn^{2+} /1000 mL • 1000 mL V_1 = 1 mg ÷ 0.65 mg/mL V_1= 1.54 mL of Zn^{2+} stock
3	Proceed with steps 8 and 9 above.	

Footnote

a. Ensure all reagents (eg, deionized water to prepare acid and base and cation solutions) have been verified as having an iron content of ≤ 0.03 mg/L.

H1.3 Determining Broth Microdilution End Points

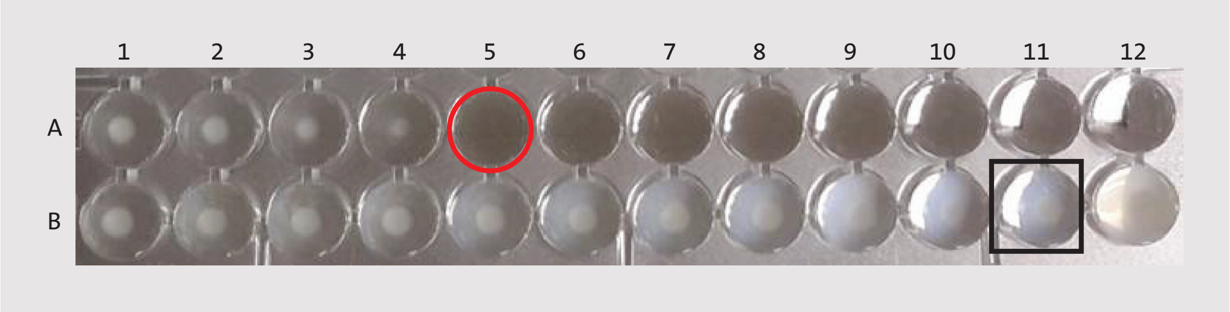
The steps for reading and interpreting broth microdilution end points for cefiderocol when tested with ID-CAMHB are listed below.

Step	Action	Comments
1	Ensure the GC well demonstrates adequate growth in the form of a button of approximately ≥ 2 mm or heavy turbidity.	Viewing devices intended to facilitate reading microdilution tests and recording results may be used as long as there is no compromise in the ability to discern growth in the wells.
2	Compare the amount of growth in the wells containing the cefiderocol with the amount of growth in the GC well containing ID-CAMHB (no antimicrobial agent). Read the MIC as the lowest concentration of cefiderocol (first clear well) where no trailing (button ≤ 1 mm) or light haziness is observed. See Figures H1–H3. If reduced growth is observed, read the MIC as the lowest concentration of cefiderocol in which the reduction of growth compared with the GC well corresponds to: • A button of approximately ≤ 1 mm (see Figure H2) or • A light haze or faint turbidity with a significant (eg, 80%) reduction compared with the GC well (see Figure H3)	Trailing growth can make end-point determination difficult. Trailing occurs most frequently with *Acinetobacter* spp. and *Pseudomonas aeruginosa*. The laboratory may wish to perform repeat testing on isolates when trailing makes it difficult to determine an end point, especially if reduced growth is followed by an increase in growth at higher concentrations. See Figure H2, panel C.

Appendix H. (Continued)

Step	Action	Comments
3	Interpret the results.	Refer to the appropriate portions of Tables 2 for breakpoints.

Abbreviations: GC, growth control; ID-CAMHB, iron-depleted cation-adjusted Mueller-Hinton broth; MIC, minimal inhibitory concentration.

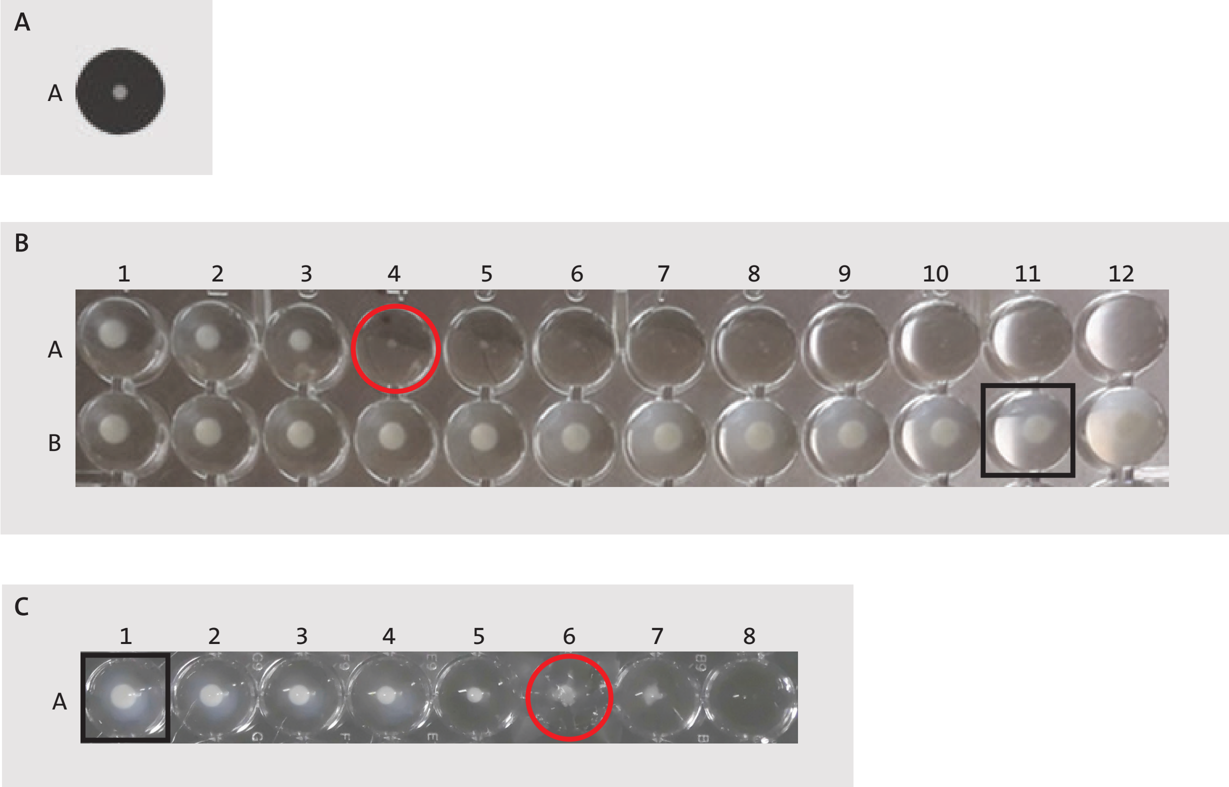


Abbreviations: GC, growth control; MIC, minimal inhibitory concentration.

Figure H1. Cefiderocol MIC Test With a Clear End Point.

The cefiderocol concentrations in wells A1 to A12 are 0.03 to 64 µg/mL. The cefiderocol MIC at 0.5 µg/mL is in well A5 (red circle). The GC well is B11 (black box).

Appendix H. (Continued)

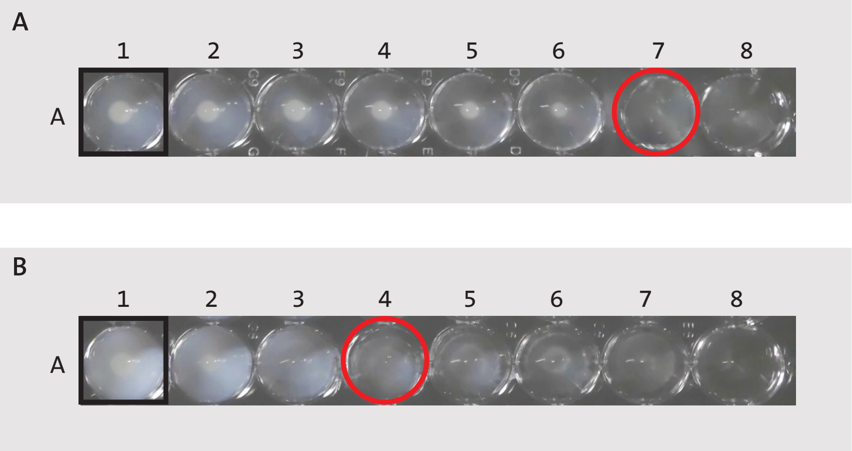


Abbreviations: GC, growth control; MIC, minimal inhibitory concentration.

Figure H2. Cefiderocol MIC Test With a Trailing End Point.

An example of a growth button with 1-mm diameter in proportion to the 7-mm diameter of a well in a 96-well MIC panel (A). MICs read at the first well corresponding to a button of ≤ 1 mm (B and C). Cefiderocol concentrations of 0.03 to 64 µg/mL in wells A1 to A12, with a cefiderocol MIC read at 0.25 µg/mL in well A4 (red circle); the GC well is B11 (black box) (B). Cefiderocol concentrations of 1 to 64 µg/mL in wells A2 to A8, with a cefiderocol MIC read at 16 µg/mL in well A6 (red circle); the GC well is A1 (black box) (C).

Appendix H. (Continued)



Abbreviations: GC, growth control; MIC, minimal inhibitory concentration.

Figure H3. Cefiderocol MIC Test With Light Haze or Faint Turbidity.

Cefiderocol concentrations of 1 to 64 µg/mL in wells A2 to A8 and MICs read at the first well corresponding to the presence of light haze or faint turbidity with a significant (eg, 80%) reduction compared with the GC well. Cefiderocol MIC read at 32 µg/mL in well A7 (red circle); the GC well is A1 (black box) (A). Cefiderocol MIC read at 4 µg/mL in well A4 (red circle); the GC well is A1 (black box) (B).

H2 Exebacase Broth Preparation and Reading Broth Microdilution Minimal Inhibitory Concentration End Points

H2.1 Calcium and Magnesium Stock Solutions

Refer to CLSI M07[1] for cation stock solution preparation.

H2.2 Exebacase Stock Solution

Refer to Table 6A for exebacase stock solution preparation.

Appendix H. (Continued)

The steps for handling exebacase stock solution are listed below.

Step	Action	Comments
1	Thaw frozen stock solution in a 25°C water bath with gentle mixing every 30 s.	Thawing should not take more than 5 min. The thawed stock solution and any subsequently prepared dilutions in CAMHB-HSD should be kept chilled in an ice bucket or refrigerated at 2 to 8°C for no more than 1 h while broth microdilution MIC panels are prepared.
2	Discard any remaining unused stock solution.	

Abbreviations: CAMHB-HSD, cation-adjusted Mueller-Hinton broth supplemented with horse serum (25% v/v) and 0.5 mM DL-dithiothreitol (pH 7.2–7.4); h, hour(s); MIC, minimal inhibitory concentration; min, minute(s); s, second(s).

H2.3 Exebacase CAMHB-HSD

The steps for preparing 1 L of cation-adjusted Mueller-Hinton broth supplemented with horse serum (25% v/v) and 0.5 mM DL-dithiothreitol (pH 7.2–7.4) (CAMHB-HSD) are listed below.

Step	Action	Comments
1	Prepare or obtain 750 mL sterile CAMHB.	CAMHB should be prepared according to manufacturer instructions or according to CLSI M07.[1]
2	Add 250 mL horse serum to 750 mL sterile CAMHB.	Final 25% v/v horse serum.
3	Remove 500 µL CAMHB.	
4	Add 500 µL DL-dithiothreitol (pH 7.2–7.4)	

Abbreviations: CAMHB, cation-adjusted Mueller-Hinton broth; pH, negative logarithm of hydrogen ion concentration.

NOTE 1: Prepared MIC panels with CAMHB-HSD and exebacase should be frozen within 15 minutes of preparation.

NOTE 2: CAMHB-HSD is used for testing exebacase against staphylococci and β-hemolytic streptococci.

NOTE 3: CAMHB-HSD does not require addition of lysed horse blood when testing β-hemolytic streptococci.

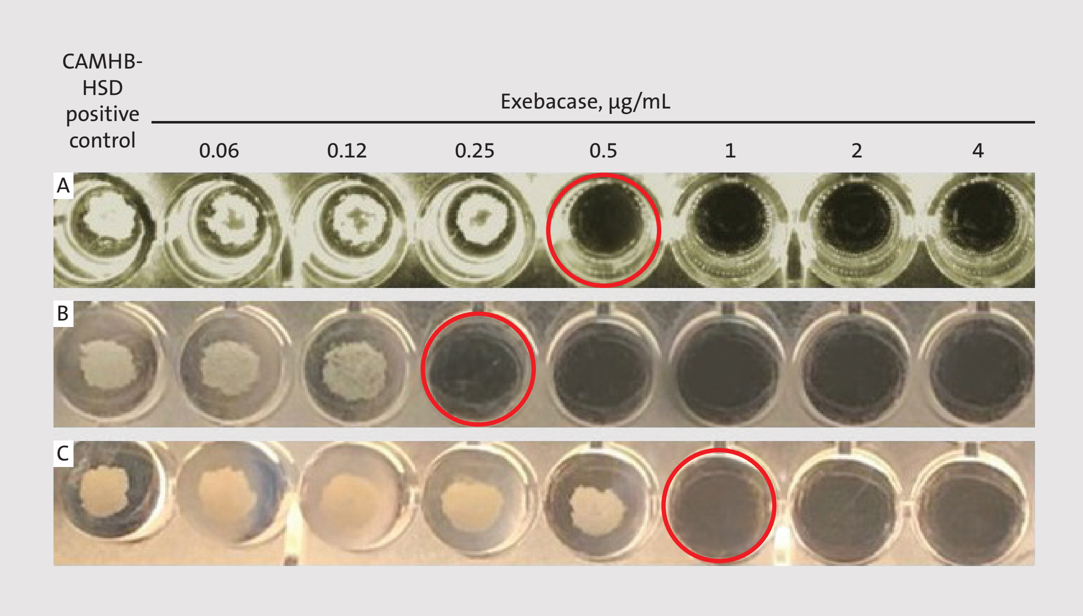
Appendix H. (Continued)

H2.4 Determining Broth Microdilution End Points

The protocols for testing and reading end points for exebacase when tested with CAMHB-HSD are listed below.

Organism Group	Incubation	End Points
Staphylococcus aureus	**Ambient conditions for 16–20 h**	**Read MIC end points as shown in Figure H4.**
SOSA	**5% CO_2 for 20–24 h**	**Read MIC end points at complete inhibition as shown in Figure H5.**
β-hemolytic streptococci	**Ambient conditions for 20–24 h**	**Read MIC end points at complete inhibition.**

Abbreviations: CO_2, carbon dioxide; h, hour(s); MIC, minimal inhibitory concentration; SOSA, staphylococci other than *Staphylococcus aureus*.

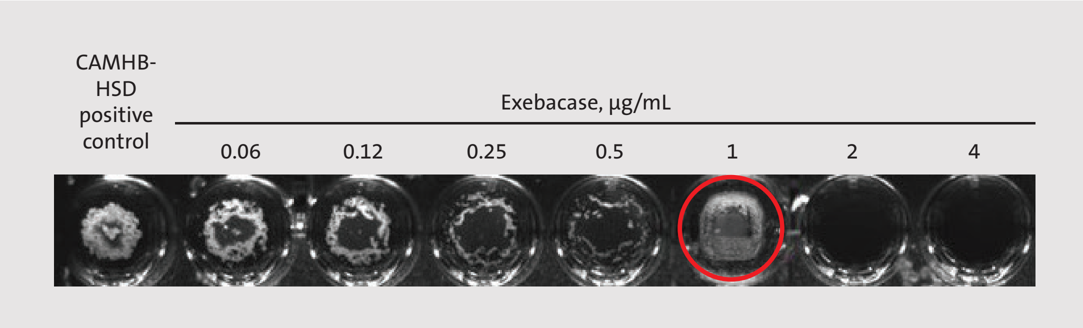


Abbreviations: CAMHB-HSD, cation-adjusted Mueller-Hinton broth supplemented with horse serum (25% v/v) and 0.5 mM DL-dithiothreitol (pH 7.2–7.4); GC, growth control; MIC, minimal inhibitory concentration; pH, negative logarithm of hydrogen ion concentration.

Figure H4. Exebacase MIC Test: *S. aureus* Incubated in Ambient Conditions for 16–20 Hours, With MICs Shown in Red Circles

Most end points are read as complete inhibition of growth compared with the GC well, eg, the MIC at 0.5 µg/mL (A). In some cases, a marked reduction in growth compared with GC is observed, eg, the MIC at 0.25 µg/mL (B) and the MIC at 1 µg/mL (C).

Appendix H. (Continued)



Abbreviations: CAMHB-HSD, cation-adjusted Mueller-Hinton broth supplemented with horse serum (25% v/v) and 0.5 mM DL-dithiothreitol (pH 7.2–7.4); CO_2, carbon dioxide; GC, growth control; MIC, minimal inhibitory concentration; pH, negative logarithm of hydrogen ion concentration; SOSA, staphylococci other than *Staphylococcus aureus*.

Figure H5. Exebacase MIC Test: SOSA Incubated in 5% CO_2, With MICs Shown in Red Circles

For SOSA, a marked reduction in growth compared with GC is frequently observed and MICs should be read at complete inhibition; the figure shows the MIC at 1 µg/mL.

NOTE: Information in boldface type is new or modified since the previous edition.

References for Appendix H

[1] CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*. 12th ed. CLSI standard M07. Clinical and Laboratory Standards Institute; 2024.

[2] Hackel MA, Tsuji M, Yamano Y, Echols R, Karlowsky JA, Sahm DF. Reproducibility of broth microdilution MICs for the novel siderophore cephalosporin, cefiderocol, determined using iron-depleted cation-adjusted Mueller-Hinton broth. *Diagn Microbiol Infect Dis*. 2019;94(4):321-325. doi:10.1016/j.diagmicrobio.2019.03.003

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Appendix I. Selection of Quality Control Strains and Quality Control Testing Frequency

Abbreviations for Appendix I

AST	antimicrobial susceptibility testing
ATCC®[a]	American Type Culture Collection
CMS	Centers for Medicare & Medicaid Services
IQCP	individualized quality control plan
MIC	minimal inhibitory concentration
NaCl	sodium chloride
pH	negative logarithm of hydrogen ion concentration
QA	quality assurance
QC	quality control

I1 Regulatory Requirements for Selection of Quality Control Strains and Quality Control Testing Frequency

The Centers for Medicare & Medicaid Services (CMS) requires laboratories in the United States to perform appropriate QC testing for antimicrobial susceptibility testing (AST) with each lot/batch or shipment of media and antimicrobial agent(s) before, or concurrent with initial use.[1] Thereafter, QC must be performed with each day of testing (subsequently referred to as “daily” QC testing). The specific QC strains required for daily QC testing are not specified by CMS. Other regulatory agencies may have alternative QC requirements.

I2 Development of an Individualized Quality Control Plan

A laboratory in the United States must develop an individualized quality control plan (IQCP) if it wishes to deviate from CMS’s daily AST QC requirement. If an IQCP is acceptable to the laboratory’s director and accreditation requirements, an IQCP can be designed to reduce AST QC frequency and to determine which QC strains to test.

When developing an IQCP, the laboratory should select QC strains to detect both system and identifiable errors. The IQCP should include data from the laboratory to support less frequent (eg, weekly, monthly) than the CMS-required daily QC testing.

The IQCP considers both QA processes (eg, equipment maintenance, laboratory procedures, personnel competency assessment) and QC (QC plans for media and reagents). The examples in CLSI M100 focus on QC plans.

Appendix I. (Continued)

I3 Resources for Development of an Individualized Quality Control Plan for Antimicrobial Susceptibility Testing

Jointly prepared materials are available that can guide the development of an IQCP for a commercial automated AST system or disk diffusion AST.[2] Additional guidance for the development of an IQCP is provided in CLSI EP23.[3]

I4 Type of Quality Control Errors

Out-of-range QC results may be due to random, identifiable, or system errors. A QC plan focuses on detecting system failures and identifiable errors while reducing repeat testing due to random errors. The troubleshooting guides in Tables 4D (disk diffusion) and 5G (minimal inhibitory concentration [MIC]) includes descriptions for likely causes of various types of AST system failures and provides suggestions for problem resolution.

Random or identifiable errors are out-of-range QC results that:

- Can be easily explained
- Correct on repeat testing with the same or a new QC strain
- Can be a result of chance and not a system failure
- Are very unlikely to affect patient results

Some examples include no growth of the QC strain, mixed culture used for QC, or incorrect QC strain tested.

Infrequent (≤ 5%) out-of-range QC results might be due to random errors. Identifiable QC errors can be due to the failure of the operator to strictly adhere to every detail of the testing procedure (eg, incorrect preparation of the McFarland standardized suspension).

System failures are out-of-range QC results that:

- Are due to a malfunction of an instrument
- Are due to defective media and/or reagents
- Do not correct with repeat testing with the same or a new QC strain
- Can affect patient results

Some examples include system malfunction (eg, issues with optical system, blocked reagent line, software), manufacturing issue with media and/or reagents (eg, incorrect concentration of drug, incorrect contents of media), or degradation of drug or media in the test system.

I5 Selection of Quality Control Strains to Quality Control Antimicrobial Agents and Specific Media Components

The manufacturer of a new lot of AST media and/or reagents must ensure the quality before release. This typically includes testing QC strains recommended by CLSI and an international reference standard[4] in addition to testing manufacturer-selected QC strains and additional analyses (eg, high-performance liquid chromatography).

Appendix I. (Continued)

CLSI and regulatory documents describe QC strains that can be used to control adequate concentrations of antimicrobial agent(s) in an AST system as well as other parameters, such as components of the media (eg, pH, Ca^{2+}, Mg^{2+}, Zn^{2+}, iron, thymidine, 2% NaCl) that have been shown to significantly affect the activity of certain antimicrobial agent–organism combinations[4] (see CLSI M100 troubleshooting guides for disk diffusion [Table 4D] and for MIC [Table 5G]).

I6 Quality Control Plans

In the user's laboratory, a QC plan involves both the selection of QC strains and the frequency of QC testing. Each new lot/batch should be tested with the appropriate QC strains before use with QC strains selected based on the antimicrobial agent(s) tested. QC test frequency should be based on experience with and performance of the AST in the user's laboratory.

Factors to consider:

- For manufacturers:
  - Lot/batch QC is focused on ensuring the quality of media and/or reagents for newly manufactured lots before release.
- For users:
  - Lot/batch QC is focused on confirming the quality of media and/or reagents for a new lot/batch in user's laboratory.
- Shipment QC is focused on confirming quality of media and/or reagents following shipping and transport.
- Routine QC is focused on confirming quality of the media and/or reagents throughout the shelf life.

Many MIC methods (eg, panels, cards) contain antimicrobial agents and media in a closed or contained system. For other MIC methods (eg, gradient diffusion strips), antimicrobial agents and media are procured as separate components of the AST. For these, lot/batch QC is recommended when either component is changed.

Disk diffusion consists of antimicrobial disks and media procured as separate components of the AST. For these, lot/batch QC is recommended when either component is changed.

Following new lot/new shipment QC, selection of QC strains and QC frequency for the same lot/new shipment and routine QC should, at a minimum, consider:

- The ability of a QC strain to detect a system problem (eg, drug deterioration) that might occur following acceptance of a lot/batch.
- The AST system manufacturer's recommendations for any instrument or operator checks.
- System failures that have been observed in the user's laboratory or previously reported in the literature.

Appendix I. (Continued)

I7 Indicators to Detect Antimicrobial Susceptibility Testing System Problems

Components of media and other test parameters that are known to affect AST results, as well as antimicrobial agents and QC strains that can be used to monitor these are described in the M100 disk diffusion and MIC troubleshooting guides (see Tables 4D and 5G) and an international reference standard.[4] Results that might be observed if these are outside of acceptable limits are also listed (eg, zone too large, MIC too low).

I8 Example Quality Control Plans: User's Laboratory

Examples for QC strain selection for a QC plan when testing commonly tested antimicrobial agents are provided in Tables I1 and I2 for disk diffusion methods and Tables I3 and I4 for MIC methods. The manufacturer's requirement, as stated in their instructions for use, and each laboratory's experience with their AST should guide use of any additional QC strains or fewer or alternative QC strains to those listed here. This might include adding one or more strains from Appendix C or other strains as identified by the laboratory.

Out-of-range QC results with a specific antimicrobial agent/QC strain combination may suggest an issue with the parameter(s) known to impact the specific AST results. Refer to M100 troubleshooting guides for disk diffusion (Table 4D) and MIC (Table 5G) and/or an international reference standard[4] for potential causes and suggested actions.

Routine user QC should, at a minimum, include QC strains that are indicators for antimicrobial agent deterioration due to exposure to elevated temperatures during shipping and/or storage. For example, imipenem and clavulanate are among the most temperature-labile antimicrobial agents. During QC testing, the observation of in-range QC results for imipenem with *Pseudomonas aeruginosa* ATCC® 27853 and *Escherichia coli* ATCC® 35218 or for amoxicillin-clavulanate with *Klebsiella pneumoniae* ATCC® 700603 would suggest these agents and any companion agents (eg, on the same panel) were transported and stored at appropriate temperatures. The QC strains used to detect certain issues can be unique to the individual antimicrobial agent. For example, *P. aeruginosa* ATCC® 27853 detects deterioration of imipenem; whereas *K. pneumoniae* ATCC® BAA-1705™ or *K. pneumoniae* ATCC® BAA-2814™ is required to confirm the quality of both components of a β-lactam combination agent, such as imipenem-relebactam.

P. aeruginosa ATCC® 27853 also can detect issues with media/reagent parameters (eg, pH, Ca^{2+}, Mg^{2+}) that are known to affect the activity of certain antimicrobial agents. *Enterococcus faecalis* ATCC® 29212 is an indicator for thymidine content when testing trimethoprim-sulfamethoxazole. During new lot production, the manufacturer performs extensive testing to ensure these parameters are controlled. Nevertheless, these factors might need to be considered when developing a QC plan in a user's laboratory.

Appendix I. (Continued)

Table I1: Example QC Strain Selection for Disk Diffusion Methods When Testing Nonfastidious Gram-Negative Organisms

Antimicrobial Agents	Manufacturer Lot QC[a]	In User's Laboratory		
		New Lot, New Shipment QC	Same Lot, New Shipment QC	Routine QC
Ampicillin	*E. coli* ATCC®[b] 25922	*E. coli* ATCC® 25922	*E. coli* ATCC® 25922	*E. coli* ATCC® 25922
Cefepime	• *E. coli* ATCC® 25922 • *P. aeruginosa* ATCC® 27853[c]	*P. aeruginosa* ATCC® 27853[c]	*P. aeruginosa* ATCC® 27853[c]	*P. aeruginosa* ATCC® 27853[c]
Cefiderocol				
Ceftriaxone				
Ciprofloxacin				
Gentamicin				
Imipenem[c]				
Tetracycline				
Tigecycline				
Tobramycin				
Trimethoprim-sulfamethoxazole	• *E. coli* ATCC® 25922 • *E. faecalis* ATCC® 29212	• *E. coli* ATCC® 25922 • *E. faecalis* ATCC® 29212	*E. coli* ATCC® 25922	*E. coli* ATCC® 25922
Amoxicillin-clavulanate[c,d]	*E.coli* ATCC® 35218[c]	*E.coli* ATCC® 35218[c]	*E.coli* ATCC® 35218[c]	*E.coli* ATCC® 35218[c]
Piperacillin-tazobactam[d]				
Ceftazidime-avibactam[d]	*K. pneumoniae* ATCC® 700603	*K. pneumoniae* ATCC® 700603	*K. pneumoniae* ATCC® 700603	*K. pneumoniae* ATCC® 700603
Ceftolozane-tazobactam[d]				
Imipenem-relebactam[c,d]	*K. pneumoniae* ATCC® BAA-1705™ or *K. pneumoniae* ATCC® BAA-2814™	*K. pneumoniae* ATCC® BAA-1705™ or *K. pneumoniae* ATCC® BAA-2814™	*K. pneumoniae* ATCC® BAA-1705™ or *K. pneumoniae* ATCC® BAA-2814™	*K. pneumoniae* ATCC® BAA-1705™ or *K. pneumoniae* ATCC® BAA-2814™
Meropenem-vaborbactam[d]				

Abbreviations: ATCC®, American Type Culture Collection; QC, quality control.

Appendix I. (Continued)

Footnotes

a. Manufacturer lot QC requires additional QC strains and analyses to ensure the quality of media and/or reagents before release.

b. ATCC® is a registered trademark of the American Type Culture Collection. Per ATCC® convention, the trademark symbol is used after "BAA" in each catalog number, in conjunction with the registered ATCC® name.

c. Antimicrobial agent is very temperature labile (clavulanate, imipenem). The QC strain–antimicrobial agent combinations listed are critical indicators for drug deterioration due to improper transport and/or storage.

d. If a QC result for a β-lactam combination agent is out-of-range (eg, zone too large) with a β-lactamase–producing QC strain (eg, *K. pneumoniae* ATCC® 700603), the QC strain might have lost its resistance plasmid during storage and this could be the cause for the out-of-range QC result. See Table 4A-2.

Table I2: Example QC Strain Selection for Disk Diffusion Methods When Testing Nonfastidious Gram-Positive Organisms

Antimicrobial Agents	Manufacturer Lot QC[a]	In User's Laboratory		
		New Lot, New Shipment QC	Same Lot, New Shipment QC	Routine QC
Ampicillin	*Staphylococcus aureus* ATCC®[b] 25923	*S. aureus* ATCC® 25923	*S. aureus* ATCC® 25923	*S. aureus* ATCC® 25923
Cefoxitin				
Ciprofloxacin				
Clindamycin				
Erythromycin				
Oxacillin				
Tetracycline				
Vancomycin				
Trimethoprim-sulfamethoxazole	• *S. aureus* ATCC® 25923 • *E. faecalis* ATCC® 29212	• *S. aureus* ATCC® 25923 • *E. faecalis* ATCC® 29212	*S. aureus* ATCC® 25923	*S. aureus* ATCC® 25923

Abbreviations: ATCC®, American Type Culture Collection; QC, quality control.

Footnotes

a. Manufacturer lot QC requires additional QC strains and analyses to ensure the quality of media and/or reagents before release.

b. ATCC® is a registered trademark of the American Type Culture Collection.

Appendix I. (Continued)

Table I3: Example QC Strain Selection for MIC Methods When Testing Nonfastidious Gram-Negative Organisms

Antimicrobial Agents	Manufacturer Lot QC[a]	In User's Laboratory		
		New Lot, New Shipment QC	Same Lot, New Shipment QC	Routine QC
Ampicillin	*E. coli* ATCC®[b] 25922	*E. coli* ATCC® 25922	*E. coli* ATCC® 25922	*E. coli* ATCC® 25922
Cefazolin				
Cefepime	• *E. coli* ATCC® 25922 • *P. aeruginosa* ATCC® 27853[c]	*P. aeruginosa* ATCC® 27853[c]	*P. aeruginosa* ATCC® 27853[c]	*P. aeruginosa* ATCC® 27853[c]
Cefiderocol				
Ceftriaxone				
Ciprofloxacin				
Gentamicin				
Imipenem[c]				
Tetracycline				
Tigecycline				
Tobramycin				
Trimethoprim-sulfamethoxazole	• *E. coli* ATCC® 25922 • *E. faecalis* ATCC® 29212	• *E. coli* ATCC® 25922 • *E. faecalis* ATCC® 29212	*E. coli* ATCC® 25922	*E. coli* ATCC® 25922
Amoxicillin-clavulanate[c,d]	*E.coli* ATCC® 35218[c] or *K. pneumoniae* ATCC® 700603[c]	*E.coli* ATCC® 35218[c] or *K. pneumoniae* ATCC® 700603[c]	*E.coli* ATCC® 35218[c] or *K. pneumoniae* ATCC® 700603[c]	*E.coli* ATCC® 35218[c] or *K. pneumoniae* ATCC® 700603[c]
Piperacillin-tazobactam[d]				
Ceftazidime-avibactam[d]	*K. pneumoniae* ATCC® 700603	*K. pneumoniae* ATCC® 700603	*K. pneumoniae* ATCC® 700603	*K. pneumoniae* ATCC® 700603
Ceftolozane-tazobactam[d]				
Imipenem-relebactam[c,d]	*K. pneumoniae* ATCC® BAA-1705™ or *K. pneumoniae* ATCC® BAA-2814™	*K. pneumoniae* ATCC® BAA-1705™ or *K. pneumoniae* ATCC® BAA-2814™	*K. pneumoniae* ATCC® BAA-1705™ or *K. pneumoniae* ATCC® BAA-2814™	*K. pneumoniae* ATCC® BAA-1705™ or *K. pneumoniae* ATCC® BAA-2814™
Meropenem-vaborbactam[d]				

Abbreviations: ATCC®, American Type Culture Collection; MIC, minimal inhibitory concentration; QC, quality control.

Appendix I. (Continued)

Footnotes

a. Manufacturer lot QC requires additional QC strains and analyses to ensure the quality of media and/or reagents before release.

b. ATCC® is a registered trademark of the American Type Culture Collection. Per ATCC® convention, the trademark symbol is used after "BAA" in each catalog number, in conjunction with the registered ATCC® name.

c. Antimicrobial agent is very temperature labile (clavulanate, imipenem). The QC strain–antimicrobial agent combinations listed are critical indicators for drug deterioration due to improper transport and/or storage.

d. If a QC result for a β-lactam combination agent is out-of-range (eg, MIC too low) with a β-lactamase–producing QC strain (eg, *K. pneumoniae* ATCC® 700603), the QC strain might have lost its resistance plasmid during storage and this could be the cause for the out-of-range QC result. See Table 5A-2.

Table I4: Example QC Strain Selection for MIC Methods when Testing Nonfastidious Gram-Positive Organisms

		In User's Laboratory		
Antimicrobial Agents	**Manufacturer Lot QC[a]**	**New Lot, New Shipment QC**	**Same Lot, New Shipment QC**	**Routine QC**
Ampicillin	• *S. aureus* ATCC®[b] 29213 • *E. faecalis* ATCC® 29212	• *S. aureus* ATCC® 29213 • *E. faecalis* ATCC® 29212	*S. aureus* ATCC® 29213 or *E. faecalis* ATCC® 29212	*S. aureus* ATCC® 29213 or *E. faecalis* ATCC® 29212
Ciprofloxacin				
Clindamycin				
Daptomycin				
Erythromycin				
Tetracycline				
Vancomycin				
Cefoxitin	*S. aureus* ATCC® 29213	*S. aureus* ATCC® 29213	*S. aureus* ATCC® 29213	*S. aureus* ATCC® 29213
Oxacillin	• *S. aureus* ATCC® 29213 • *E. faecalis* ATCC® 29212	*S. aureus* ATCC® 29213	*S. aureus* ATCC® 29213	*S. aureus* ATCC® 29213
Trimethoprim-sulfamethoxazole	• *S. aureus* ATCC® 29213 • *E. faecalis* ATCC® 29212	• *S. aureus* ATCC® 29213 • *E. faecalis* ATCC® 29212	*S. aureus* ATCC® 29213	*S. aureus* ATCC® 29213

Abbreviations: ATCC®, American Type Culture Collection; MIC, minimal inhibitory concentration; QC, quality control.

Footnotes

a. Manufacturer lot QC requires additional QC strains and analyses to ensure the quality of media and/or reagents before release.

b. ATCC® is a registered trademark of the American Type Culture Collection.

Appendix I. (Continued)

NOTE: Information in boldface type is new or modified since the previous edition.

Footnote for Appendix I

a. ATCC® is a registered trademark of the American Type Culture Collection. Per ATCC® convention, the trademark symbol is used after "BAA" in each catalog number, in conjunction with the registered ATCC® name.

References for Appendix I

[1] Centers for Medicare & Medicaid Services, US Department of Health and Human Services. *Part 493—Laboratory Requirements; Standard: Bacteriology* (Codified at 42 CFR §493.1261). Office of the Federal Register; published annually.

[2] American Society for Microbiology. Protocols: individualized quality control plan (IQCP). Accessed 15 October 2024. https://asm.org/protocols/individualized-quality-control-plan-iqcp

[3] CLSI. *Laboratory Quality Control Based on Risk Management*. 2nd ed. CLSI guideline EP23. Clinical and Laboratory Standards Institute; 2023.

[4] ISO. *Clinical laboratory testing – Criteria for acceptable lots of dehydrated Mueller-Hinton agar and broth for antimicrobial susceptibility testing*. ISO/TS 16782. International Organization for Standardization; 2016.

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Glossary I (Part 1). β-Lactams: Class and Subclass Designations and Generic Names

In the late 1990s, several authorities were consulted to construct the glossary. The intention was to include all agents that appeared in CLSI M100, along with related agents available for human use. Since that time, agents have been added to the glossary as they were introduced to CLSI, and they do not need to be FDA cleared to be included. It cannot be assumed that the list is exhaustive, and some agents are no longer available for human use.

Antimicrobial Class	Antimicrobial Subclasses		Agents Included; Generic Names
Penicillins	Penicillinase-labile penicillins[a]	Penicillin	Penicillin
		Aminopenicillins	Amoxicillin
			Ampicillin
		Carboxypenicillins	Carbenicillin
			Ticarcillin
		Ureidopenicillins	Azlocillin
			Piperacillin
	Penicillinase-stable penicillins[b]		Cloxacillin
			Dicloxacillin
			Nafcillin
			Oxacillin
	Amdinocillin		Mecillinam

Glossary I (Part 1). (Continued)

Antimicrobial Class	Antimicrobial Subclasses	Agents Included; Generic Names
β-Lactam combination agents		Amoxicillin-clavulanate Ampicillin-sulbactam Aztreonam-avibactam Aztreonam-nacubactam (1:1) Cefepime-enmetazobactam (4:1) Cefepime-nacubactam (1:1) Cefepime-taniborbactam Cefepime-tazobactam (1:1) Cefepime-zidebactam Ceftaroline-avibactam Ceftazidime-avibactam Ceftibuten-avibactam Ceftibuten-ledaborbactam **Ceftibuten-xeruborbactam** Ceftolozane-tazobactam Imipenem-funobactam Imipenem-relebactam Meropenem-nacubactam (1:1) Meropenem-vaborbactam Meropenem-xeruborbactam Piperacillin-tazobactam Sulbactam-durlobactam Ticarcillin-clavulanate
Cephems (parenteral)	Cephalosporins I[c]	Cefazolin Cephalothin Cephapirin Cephradine
	Cephalosporins II[c]	Cefamandole Cefonicid Cefuroxime (parenteral)

Glossary I (Part 1). (Continued)

Antimicrobial Class	Antimicrobial Subclasses	Agents Included; Generic Names
Cephems (parenteral) (Continued)	Cephalosporins III[c]	Cefoperazone Cefotaxime Ceftazidime Ceftizoxime Cetriaxone
	Cephalosporins IV[c]	Cefepime Cefpirome
	Cephalosporins with anti-MRSA activity	Ceftaroline Ceftobiprole
	Cephamycins	Cefmetazole Cefotetan Cefoxitin
	Oxacephem	Moxalactam
	Siderophore cephalosporin	Cefiderocol
Cephems (oral)	Cephalosporins	Cefaclor Cefadroxil Cefdinir Cefditoren Cefetamet Cefixime Cefpodoxime Cefprozil Ceftibuten Cefuroxime (oral) Cephalexin Cephradine
	Carbacephem	Loracarbef

Glossary I (Part 1). (Continued)

Antimicrobial Class	Antimicrobial Subclasses	Agents Included; Generic Names
Monobactams		Aztreonam
Penems	Carbapenems	Biapenem Doripenem Ertapenem Imipenem Meropenem Razupenem Tebipenem
	Penems	Faropenem Sulopenem

Abbreviations: ESBL, extended-spectrum β-lactamase; FDA, US Food and Drug Administration; MRSA, methicillin (oxacillin)-resistant *Staphylococcus aureus*.

Footnotes

a. Hydrolyzed by staphylococcal penicillinase.

b. Not hydrolyzed by staphylococcal penicillinase.

c. Cephalosporins I, II, III, and IV are sometimes referred to as first-, second-, third-, and fourth-generation cephalosporins, respectively. Cephalosporins III and IV are also referred to as "extended-spectrum cephalosporins." This does not imply activity against ESBL-producing gram-negative bacteria.

NOTE: Information in boldface type is new or modified since the previous edition.

Glossary I (Part 2). Non–β-Lactams: Class and Subclass Designations and Generic Names

In the late 1990s, several authorities were consulted to construct the glossary. The intention was to include all agents that appeared in CLSI M100, along with related agents available for human use. Since that time, agents have been added to the glossary as they were introduced to CLSI, and they do not need to be FDA cleared to be included. It cannot be assumed that the list is exhaustive, and some agents are no longer available for human use.

Antimicrobial Class	Antimicrobial Subclasses	Agents Included; Generic Names
Aminocyclitols		Spectinomycin
Aminoglycosides		Amikacin Gentamicin Kanamycin Netilmicin Plazomicin Streptomycin Tobramycin
Aminoglycoside-fosfomycin		Amikacin-fosfomycin
Ansamycins	Rifamycins	Rifabutin Rifapentine Rifampin Rifaximin
Lysins	Lysin with antistaphylococcal activity	Exebacase
Folate pathway antagonists	Dihydrofolate reductase inhibitors	Iclaprim Sulfonamides Trimethoprim Trimethoprim-sulfamethoxazole
	Sulfonamides	Sulfamethoxazole Sulfisoxazole
	Combination	Trimethoprim-sulfamethoxazole
Fosfomycins		Fosfomycin

Glossary I (Part 2). (Continued)

Antimicrobial Class	Antimicrobial Subclasses	Agents Included; Generic Names
Glycopeptides	Glycopeptide	Vancomycin
	Lipoglycopeptides	Dalbavancin Oritavancin Teicoplanin Telavancin
	Lipoglycodepsipeptide	Ramoplanin
Lincosamides		Clindamycin Lincomycin
Lipopeptides		Daptomycin Surotomycin
	Polymyxins	Colistin Polymyxin B Upleganan
Macrocyclic lactone		Fidaxomicin
Macrolides		Azithromycin Clarithromycin Dirithromycin Erythromycin
	Fluoroketolide	Solithromycin
	Ketolides	Nafithromycin Telithromycin
Nitroheterocyclics	Nitrofuran	Nitrofurantoin
	Nitroimidazoles	Metronidazole Secnidazole Tinidazole
	Thiazolides	Nitazoxanide Tizoxanide
Oxazolidinones		Linezolid Tedizolid

Glossary I (Part 2). (Continued)

Antimicrobial Class	Antimicrobial Subclasses	Agents Included; Generic Names
Peptides	Magainin	Pexiganan
	Tethered macrocyclic	**Zosurabalpin**
Phenicols		Chloramphenicol Thiamphenicol
Pleuromutilins		Lefamulin Retapamulin
Pseudomonic acid		Mupirocin
Quinolones		Cinoxacin Garenoxacin Nalidixic acid
	Benzoquinolizine	Levonadifloxacin
	Fluoroquinolones	Besifloxacin Ciprofloxacin Clinafloxacin Delafloxacin Enoxacin Finafloxacin Fleroxacin Gatifloxacin Gemifloxacin Grepafloxacin Levofloxacin Lomefloxacin Moxifloxacin Norfloxacin Ofloxacin Ozenoxacin Pefloxacin Sparfloxacin Trovafloxacin Ulifloxacin (prulifloxacin)

Glossary I (Part 2). (Continued)

Antimicrobial Class	Antimicrobial Subclasses	Agents Included; Generic Names
Quinolonyl oxazolidinone		Cadazolid
Spiropyrimidinetrione		Zoliflodacin
Steroid	Fusidane	Fusidic acid
Streptogramins		Quinupristin-dalfopristin
Tetracyclines		Doxycycline Minocycline Tetracycline
	Fluorocycline	Eravacycline
	Glycylcycline	Tigecycline
	Aminomethylcycline	Omadacycline
Triazaacenaphthylene		Gepotidacin

Abbreviation: FDA, US Food and Drug Administration.

NOTE: Information in boldface type is new or modified since the previous edition.

Glossary II. Antimicrobial Agent Abbreviations, Routes of Administration, and Drug Class

In the late 1990s, several authorities were consulted to construct the glossary. The intention was to include all agents that appeared in CLSI M100, along with related agents available for human use. Since that time, agents have been added to the glossary as they were introduced to CLSI, and they do not need to be FDA cleared to be included. It cannot be assumed that the list is exhaustive, and some agents are no longer available for human use.

Antimicrobial Agent	Abbreviations[a,b]		Routes of Administration[c]				Drug Class or Subclass
	CLSI Recommended	In Use	PO	IM	IV	Topical	
Amikacin	AN	AN, AK, Ak, AMI, AMK, AKN		X	X		Aminoglycoside
Amikacin-fosfomycin	AKF	AKF	X[d]				Aminoglycoside-fosfomycin
Amoxicillin	AMX	AMX, Amx, AMOX, AC, AML, A	X		X		Penicillin
Amoxicillin-clavulanate	AMC	AMC, Amc, A/C, AUG, Aug, XL, AML	X				β-Lactam combination agent
Ampicillin	AM	AM, Am, AMP, AP	X	X	X		Penicillin
Ampicillin-sulbactam	SAM	SAM, A/S, AMS, AB			X		β-Lactam combination agent
Azithromycin	AZM	AZM, Azi, AZI, AZ, ATH	X		X		Macrolide
Azlocillin	AZL	AZ, Az, AZL		X	X		Penicillin
Aztreonam	ATM	ATM, AZT, Azt, AT, AZM			X		Monobactam
Aztreonam-avibactam	AZA	AZA			X		β-Lactam combination agent
Aztreonam-nacubactam	ANC	ANC			X		β-Lactam combination agent
Besifloxacin	BES	BES				X	Fluoroquinolone
Biapenem	BPM	BPM			X		Carbapenem
Cadazolid	CDZ	CDZ	X				Quinolonyl oxazolidinone
Carbenicillin (indanyl salt) Carbenicillin	CB	CB, Cb, BAR, CAR, CRB, PY	X	X	X		Penicillin
Cefaclor	CEC	CEC, CCL, Cfr, FAC, CF, CFC	X				Cephem
Cefadroxil	CFR	CFR, FAD, CDX	X				Cephem
Cefamandole	MA	MA, CM, Cfm, FAM, CMD		X	X		Cephem
Cefazolin	CZ	CZ, CFZ, Cfz, FAZ, KZ, CZN		X	X		Cephem
Cefdinir	CDR	CDR, Cdn, DIN, CD, CFD	X				Cephem
Cefditoren	CDN	CDN, DIT, FD	X				Cephem
Cefepime	FEP	FEP, Cpe, PM, CPM		X	X		Cephem
Cefepime-enmetazobactam	FPE	FPE			X		β-Lactam combination agent

Glossary II. (Continued)

Antimicrobial Agent	Abbreviations[a,b]		Routes of Administration[c]				Drug Class or Subclass
	CLSI Recommended	In Use	PO	IM	IV	Topical	
Cefepime-nacubactam	FNC	FNC			X		β-Lactam combination agent
Cefepime-taniborbactam	FTB	FTB			X		β-Lactam combination agent
Cefepime-tazobactam	FPT	FPT			X		β-Lactam combination agent
Cefepime-zidebactam	FPZ	FPZ			X		β-Lactam combination agent
Cefetamet	CAT	CAT, FET	X				Cephem
Cefiderocol	FDC	FDC			X		Siderophore β-lactam
Cefixime	CFM	CFM, FIX, Cfe, IX	X				Cephem
Cefmetazole	CMZ	CMZ, CMZS, CMT, Cmz		X	X		Cephem
Cefonicid	CID	CID, Cfc, FON, CPO		X	X		Cephem
Cefoperazone	CFP	CFP, Cfp, CPZ, PER, FOP, CP		X	X		Cephem
Cefotaxime	CTX	CTX, TAX, Cft, FOT, CT		X	X		Cephem
Cefotetan	CTT	CTT, CTN, Ctn, CTE, TANS, CN		X	X		Cephem
Cefoxitin	FOX	FOX, CX, Cfx, FX		X	X		Cephem
Cefpirome	CPO	CPO, CPR, CR		X	X		Cephem
Cefpodoxime	CPD	CPD, Cpd, POD, PX	X				Cephem
Cefprozil	CPR	CPR, CPZ, FP	X				Cephem
Ceftaroline	CPT	CPT, Cpt, CTR			X		Cephem
Ceftaroline-avibactam	CPA	CPA			X		β-Lactam combination agent
Ceftazidime	CAZ	CAZ, Caz, TAZ, TZ		X	X		Cephem
Ceftazidime-avibactam	CZA	CZA			X		β-Lactam combination agent
Ceftibuten	CTB	CTB, TIB, CB, CFB, CFT	X				Cephem
Ceftibuten-avibactam	CBA	CBA	X				β-Lactam combination agent
Ceftibuten-ledaborbactam	CLB	CLB	X				β-Lactam combination agent
Ceftibuten-xeruborbactam	**CBX**	**CBX**	**X**				**β-Lactam combination agent**
Ceftizoxime	ZOX	ZOX, CZX, CZ, Cz, CTZ, TIZ		X	X		Cephem
Ceftobiprole	BPR	BPR			X		Cephem
Ceftolozane-tazobactam	CT	CT, C/T, CXT, CLT			X		β-Lactam combination agent

Glossary II. (Continued)

Antimicrobial Agent	Abbreviations[a,b]		Routes of Administration[c]				Drug Class or Subclass
	CLSI Recommended	In Use	PO	IM	IV	Topical	
Ceftriaxone	CRO	CRO, CTR, FRX, Cax, AXO, TX		X	X		Cephem
Cefuroxime (oral) Cefuroxime (parenteral)	CXM	CXM, CFX, ROX, Crm, FUR, XM	X	X	X		Cephem
Cephalexin	CN	CN, LEX, CFL, CL, CFX	X				Cephem
Cephalothin	CF	CF, Cf, CR, CL, CEP, CE, KF, CEF			X		Cephem
Cephapirin	CP	CP, HAP		X	X		Cephem
Cephradine	RAD	RAD, CH, CED, CE	X				Cephem
Chloramphenicol	C	C, CHL, CL	X		X		Phenicol
Cinoxacin	CIN	CIN, Cn	X				Quinolone
Ciprofloxacin	CIP	CIP, Cp, CI	X		X		Fluoroquinolone
Clarithromycin	CLR	CLR, CLM, CLA, Cla, CH	X				Macrolide
Clinafloxacin	CLX	CFN, CLX, LF, CFL	X		X		Fluoroquinolone
Clindamycin	CM	CC, CM, CD, Cd, CLI, DA	X	X	X		Lincosamide
Cloxacillin	CLO	CX, Clx, CLO, OB, OX	X	X	X		Penicillin
Colistin	CL	CL, CS, CT, CI, CO, COL			X		Lipopeptide
Dalbavancin	DAL	DAL			X		Lipoglycopeptide
Daptomycin	DAP	DAP, Dap, DPC			X		Lipopeptide
Delafloxacin	DLX	DLX, DFX	X		X		Fluoroquinolone
Dicloxacillin	DX	DX, DIC	X				Penicillin
Dirithromycin	DTM	DTM, DT, DIR	X				Macrolide
Doripenem	DOR	DOR, Dor			X		Carbapenem
Doxycycline	DO	DO, DOX, DC, DOXY, D, DX, Dox, DXT	X		X		Tetracycline
Enoxacin	ENX	ENX, Enx, ENO, ENOX, ENO(F)	X				Fluoroquinolone
Ertapenem	ETP	ETP, Etp		X	X		Carbapenem
Eravacycline	ERV	ERV	X		X		Fluorocycline
Erythromycin	E	E, ERY, EM	X		X		Macrolide
Exebacase	EXE	EXE			X		Antistaphylococcal lysin

Glossary II. (Continued)

Antimicrobial Agent	Abbreviations[a,b]		Routes of Administration[c]				Drug Class or Subclass
	CLSI Recommended	In Use	PO	IM	IV	Topical	
Faropenem	FPM	FAR, FARO, FPM, Faro	X				Penem
Fidaxomicin	FDX	FDX	X				Macrocyclic
Finafloxacin	FIN	FIN	X		X	X	Fluoroquinolone
Fleroxacin	FLE	FLE, Fle	X		X		Fluoroquinolone
Fosfomycin	FOS	FOS, FF, FO, FM, Fos	X				Fosfomycin
Fusidic acid	FA	FA, FC, FUS, FD, FU, FAD	X		X	X	Steroidal
Garenoxacin	GRN	GRN, Grn	X		X		Quinolone
Gatifloxacin	GAT	GAT, Gat, GA, GFLX	X		X		Fluoroquinolone
Gemifloxacin	GEM	GEM, Gem	X				Fluoroquinolone
Gentamicin Gentamicin synergy	GM	GM, Gm, CN, GEN GM500, HLG, Gms, GHLR, GMS		X	X		Aminoglycoside
Gepotidacin	GEP	GEP	X		X		Triazaacenaphthylene
Grepafloxacin	GRX	GRX, Grx, GRE, GP	X				Fluoroquinolone
Iclaprim	ICL	ICL, IP			X		Folate pathway antagonist
Imipenem	IPM	IPM, IMI, Imp, IP			X		Carbapenem
Imipenem-funobactam	IPF	IPF			X		β-Lactam combination agent
Imipenem-relebactam	IMR	IMR, IPR, I/R			X		β-Lactam combination agent
Kanamycin	K	K, KAN, HLK, KM		X	X		Aminoglycoside
Lefamulin	LMU	LMU	X		X		Pleuromutilin
Levofloxacin	LVX	LVX, Lvx, LEV, LEVO, LE	X		X		Fluoroquinolone
Levonadifloxacin	LND	LND			X		Benzoquinolizine
Lincomycin	LIN	L, Lin, LIN, MY		X	X		Lincosamide
Linezolid	LZD	LNZ, LZ, LZD, Lzd	X		X		Oxazolidinone
Lomefloxacin	LOM	LOM, Lmf, LFLX, LOMX	X				Fluoroquinolone
Loracarbef	LOR	LOR, Lor	X				Cephem
Mecillinam	MEC	MEC, Mec, MM, MEL	X				Penicillin
Meropenem	MEM	MEM, Mer, MERO, MRP, MP			X		Carbapenem

Glossary II. (Continued)

Antimicrobial Agent	Abbreviations[a,b]		Routes of Administration[c]				Drug Class or Subclass
	CLSI Recommended	In Use	PO	IM	IV	Topical	
Meropenem-nacubactam	MNC	MNC			X		β-Lactam combination agent
Meropenem-vaborbactam	MEV	MEV			X		β-Lactam combination agent
Meropenem-xeruborbactam	XEM	XEM			X		β-Lactam combination agent
Methicillin	ME	ME, MET, DP		X	X		Penicillin
Metronidazole	MET	MET, MTZ, MZ, MRD, MTR	X		X		Nitroimidazole
Minocycline	MI	MI, MIN, Min, MN, MNO, MC, MH	X		X		Tetracycline
Moxalactam	MOX	MOX, Mox		X	X		Cephem
Moxifloxacin	MXF	MXF, Mxf, MX	X		X		Fluoroquinolone
Mupirocin	MUP	MUP, MOP, MU, Mup, PUM				X	Pseudomonic acid
Nafcillin	NF	NF, NAF, Naf		X	X		Penicillin
Nafithromycin	ZMK	ZMK, ZWK	X				Ketolide
Nalidixic acid	NA	NA, NAL	X				Quinolone
Netilmicin	NET	NET, Nt, NC		X	X		Aminoglycoside
Nitazoxanide	NIT	NIT	X				Thiazolide
Nitrofurantoin	FM	FM, F/M, FD, Fd, FT, NIT, NI, F	X				Nitrofuran
Norfloxacin	NX	NX, NOV, NV, NO	X				Fluoroquinolone
Novobiocin	NB	NB				X	Aminocoumarin
Ofloxacin	OFL	OFL, OFX, Ofl, OF	X	X	X		Fluoroquinolone
Omadacycline	OMC	OMC	X		X		Tetracycline
Oritavancin	ORI	ORI			X		Lipoglycopeptide
Oxacillin	OX	OX, Ox, OXS, OXA	X	X	X		Penicillin
Ozenoxacin	OZN	OZN				X	Fluoroquinolone
Pefloxacin	PEF	PEF, PF, Pef, PE					Fluoroquinolone
Penicillin	P	P, PEN, PV, PG	X	X	X		Penicillin
Pexiganan	PEX	PEX, P/N				X	Peptide
Piperacillin	PIP	PIP, PI, PP, Pi, PRL		X	X		Penicillin
Piperacillin-tazobactam	TZP	TZP, PTZ, P/T, PTc			X		β-Lactam combination agent

Glossary II. (Continued)

Antimicrobial Agent	Abbreviations[a,b]		Routes of Administration[c]				Drug Class or Subclass
	CLSI Recommended	In Use	PO	IM	IV	Topical	
Plazomicin	PLZ	PLZ			X		Aminoglycoside
Polymyxin B	PB	PB, POL, PO			X		Lipopeptide
Quinupristin-dalfopristin	SYN	SYN, Syn, QDA, RP, QDF			X		Streptogramin
Ramoplanin	RAM	RAM	X				Lipoglycodepsipeptide
Razupenem	RZM	RZ, RZM			X		Carbapenem
Rifampin	RA	RA, RIF, Rif, RI, RD, RP, RFP	X		X		Ansamycin
Rifamycin	RIF	RF, RIF	X		X		Ansamycin
Rifapentine	RPT	RPT				X	Pleuromutilin
Rifaximin	RFX	RFX	X				Ansamycin
Secnidazole	SEC	SEC	X				Nitroimidazole
Solithromycin	SOL	SOL	X		X	X	Fluoroketolide
Sparfloxacin	SPX	SPX, Sfx, SPX, SO, SPFX	X				Fluoroquinolone
Spectinomycin	SPT	SPT, SPE, SC, SP, SH, SPC		X	X		Aminocyclitol
Streptomycin Streptomycin synergy	STS	STS, S, STR, StS, SM, ST2000, HLS, SHLR		X	X		Aminoglycoside
Sulbactam-durlobactam	SUD	SUD, SUL			X		β-Lactam combination agent
Sulfonamides	SSS	G, SSS, S3	X		X		Folate pathway antagonist (some PO only)
Sulopenem	SLP	SLP, SPM	X		X		Penem
Surotomycin	SUR	SUR	X				Lipopeptide
Tebipenem	TBP	TBP	X				Carbapenem
Tedizolid	TZD	TZD	X		X		Oxazolidinone
Teicoplanin	TEC	TEC, TPN, Tei, TEI, TP, TPL		X	X		Lipoglycopeptide
Telavancin	TLV	TLV, TLA			X		Lipoglycopeptide
Telithromycin	TEL	TEL	X				Ketolide
Tetracycline	TE	TE, Te, TET, TC	X		X		Tetracycline
Thiamphenicol	TP	TP	X	X	X		Phenicol

Glossary II. (Continued)

Antimicrobial Agent	Abbreviations[a,b]		Routes of Administration[c]				Drug Class or Subclass
	CLSI Recommended	In Use	PO	IM	IV	Topical	
Ticarcillin	TIC	TIC, TC, TI, Ti		X	X		Penicillin
Ticarcillin-clavulanate	TIM	TIM, Tim, T/C, TCC, TLc, TTC			X		β-Lactam combination agent
Tigecycline	TGC	TGC, Tgc			X		Glycylcycline
Tinidazole	TNZ	TNZ	X				Nitroimidazoles
Tinoxanide	TIN	TIN	X				Thiazolide
Tobramycin	TM	TM, NN, TO, To, TOB, TN		X	X		Aminoglycoside
Trimethoprim	TMP	TMP, T, TR, W, TM	X				Folate pathway antagonist
Trimethoprim-sulfamethoxazole	SXT	SXT, SxT, T/S, TS, COT	X		X		Folate pathway antagonist
Trospectomycin	TBR	TBR		X	X		Aminocyclitol
Trovafloxacin	TRO	TVA, Tva, TRV, TV, TRO	X		X		Fluoroquinolone
Ulifloxacin (prulifloxacin)	PRU	PRU, ULI	X				Fluoroquinolone
Upleganan	UPL	UPL			X		Lipopeptide
Vancomycin	VA	VA, Va, VAN, VCM	X		X		Glycopeptide
Zoliflodacin	ZFD	ZFD	X				Spiropyriminetrione
Zosurabalpin	**ZAB**	**ZAB**			**X**		**Peptide**

Abbreviations: AST, antimicrobial susceptibility testing; FDA, US Food and Drug Administration; IM, intramuscular; IV, intravenous; PO, oral.

Footnotes

a. Abbreviations assigned to one or more diagnostic products in the United States. If no diagnostic product is available, abbreviation is that of the manufacturer.

b. Abbreviations used by AST device manufacturers may differ from those recommended by CLSI.

c. As available in the United States.

d. Amikacin-fosfomycin is aerosolized and inhaled.

NOTE: Information in boldface type is new or modified since the previous edition.

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Glossary III. List of Identical Abbreviations Used for More Than One Antimicrobial Agent in US Diagnostic Products

In the late 1990s, several authorities were consulted to construct the glossary. The intention was to include all agents that appeared in CLSI M100, along with related agents available for human use. Since that time, agents have been added to the glossary as they were introduced to CLSI, and they do not need to be FDA cleared to be included. It cannot be assumed that the list is exhaustive, and some agents are no longer available for human use.

Abbreviations	Antimicrobial Agents for Which Respective Abbreviations Are Used
AZ	Azithromycin, azlocillin
AZM	Azithromycin, aztreonam
CB, Cb	Ceftibuten, carbenicillin
CD, Cd	Clindamycin, cefdinir
CDN, Cdn	Cefdinir, cefditoren
CF, Cf	Cefaclor, cephalothin
CFM, Cfm	Cefixime, cefamandole
CFR, Cfr	Cefaclor, cefadroxil
CFX, Cfx	Cefoxitin, cefuroxime
CH	Clarithromycin, cephradine
CL	Cephalothin, chloramphenicol
CLX, Clx	Clinafloxacin, cloxacillin
CM	Clindamycin, cefamandole
CN, Cn	Cephalexin, cefotetan, cinoxacin, gentamicin
CP, Cp	Cephapirin, cefoperazone, ciprofloxacin
CPR	Cefpirome, cefprozil
CPZ	Cefprozil, cefoperazone
CT	Ceftolozane-tazobactam, colistin
CZ, Cz	Ceftizoxime, cefazolin
DX	Doxycycline, dicloxacillin
FO	Fleroxacin, fosfomycin
NIT	Nitazoxanide, nitrofurantoin
TC	Tetracycline, ticarcillin
TM	Tobramycin, trimethoprim

Abbreviation: FDA, US Food and Drug Administration.

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The Quality Management System Approach

Clinical and Laboratory Standards Institute (CLSI) subscribes to a quality management system (QMS) approach in the development of standards and guidelines that facilitates project management, defines a document structure using a template, and provides a process to identify needed documents. The QMS approach applies a core set of "quality system essentials" (QSEs), basic to any organization, to all operations in any health care service's path of workflow (ie, operational aspects that define how a particular product or service is provided). The QSEs provide the framework for delivery of any type of product or service, serving as a manager's guide. The QSEs are:

- Organization and Leadership
- Customer Focus
- Facilities and Safety Management
- Personnel Management
- Supplier and Inventory Management
- Equipment Management
- Process Management
- Documents and Records Management
- Information Management
- Nonconforming Event Management
- Assessments
- Continual Improvement

The QSEs covered by CLSI M100 and its related CLSI documents are available on the CLSI website: https://clsi.org/qse

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PRINT ISBN 978-1-68440-262-5

ELECTRONIC ISBN 978-1-68440-263-2

CLSI M100-Ed35